Parturition in the pig: relationships between pain, stress and maternal behaviour

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Abstract

The thesis examines the relationships between pain, stress and behaviour of the pig around parturition. This includes the effects of pregnancy and parturition on maternal nociception, and environmental restriction on stress physiology and behaviour. The main findings are:

1. Late pregnancy and parturition in the pig is associated with an endogenous analgesic system which is, at least in part, mediated via endogenous opioids.

2. Passivity and inactivity are major components of maternal behaviour in the pig and are suggested to be indicative of good maternal care in this species. The thesis provides evidence of an opioid mediation of maternal behaviour which could arise through several potential routes, namely: the action of the analgesic system, general sedation, the inhibition of oxytocin release.

3. A rise in plasma cortisol, ACTH and β-endorphin concentrations were found in pre-parturient gilts housed in both straw bedded pens and conventional farrowing crates. However crates caused further stimulation of the HPA axis reflecting thwarting of nestbuilding behaviour in this restrictive environment.

4. The farrowing crate did not cause further HPA activity during the expulsive phase which may reflect the inactivity of the pig at this time. A rise in plasma cortisol was found as the expulsive phase progressed irrespective of environment however the thesis found that the expulsion of a piglet does not appear to play a major role in this.

Overall the thesis has realised a better understanding of parturition in the pig by relating the physiology and behaviour of the pig at this time. The possibility of maternal pain influencing the progress of parturition and maternal behaviour is discussed in relation to possible mechanisms by which this may occur. The thesis has also highlighted welfare implications regarding the use of farrowing crates, and provides information which may be used when considering changes to housing for parturient pigs.

I hereby declare that this thesis is of my own composition, and that all assistance has been duly acknowledged. The results presented herein have not previously been submitted for any other degree or qualification

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Chapter 1

General Introduction

1.1 Introduction

Few studies of the maternal behaviour of the pig in natural environments have been carried out. However the available information suggests that the behaviour of wild sows at this time is similar to that seen in feral and free-ranging pigs (Jensen et al., 1986). Due to the increase in intensification and selection over the past 40 years a quantitative description of maternal behaviour is required to investigate the impact that this has had on the behaviour of the pig at this time.

1.2 Behaviour around farrowing

1.2.1 Free-ranging

Free-ranging female pigs in late pregnancy remain with their social group until approximately 24 hours before farrowing at which time they separate from the group in search of a suitable nesting site (Jensen et al., 1986). A study by Jensen (1989) found that most nest sites tended to be in small wooded areas and were outwith the normal home ranges. The choice of this type of area may reduce the risk of detection by predators and give protection from adverse weather conditions. The isolation from the rest of the social group may reduce trampling of piglets by other adults, prevent suckling by other older piglets and therefore allow bonding between the sow and her piglets (Jensen, 1989).

Once the nest site is selected the sow begins to construct her nest. By pawing at the ground a hollow is formed into which the sow places grass and branches which she has carried to the nest site in her mouth. She may then push the material with her nose to the outside of the hollow constructing perimeter walls. Sows have been

observed to lie in the nest but get up several times to continue nest-building (Jensen, 1986). Within the last 90 minutes before farrowing the sow spends a large proportion of time lying in the nest at which time abdominal straining can be seen (Signoret et al., 1975).

During the expulsive phase free-ranging sows are generally in lateral recumbency (Petersen et al., 1990) which allows full udder exposure as a source of warmth and nutrition for piglets (Fraser, 1984). The extremely precocial piglets will get to the udder and normally start to suckle within 30-45 minutes from birth (Petersen et al., 1990). The requirement for warmth is paramount as the sow, unlike many other mammals, does not lick and therefore remove fluids covering the body of the young (Jones, 1966b, Petersen et al., 1990). Milk is available from the udder during the 12 hours following birth and involves individual suckling, however subsequently suckling becomes more cyclical (approximately every hour) and synchronised with milk being let down for only 10-20 seconds (Lewis and Hurnik, 1985).

During the two days following farrowing the sow spends around 90% of the time in the nest, leaving mainly to forage (Stangel and Jensen, 1991). The sow and litter then occupy the nest until around 10 days post-farrowing at which time they leave the nest and rejoin the herd (Stangel and Jensen, 1991, Dellmeier and Friend, 1992). Once introduced to the group the piglets interact with other young but continue to rest with their littermates (Jensen, 1986). The distance between the mother and young increases with time and the piglets are eventually weaned at around 14-17 weeks of age (Jensen, 1986).

1.2.2 Commercial environments

The farrowing crate was introduced in the 1960's to reduce piglet mortality as a result of overlying and to benefit the producer in that they provided protection for the stockperson, reduced space requirements and allowed easier waste disposal (Cronin et al., 1991). However, the farrowing crate design has received criticism due to its potential to compromise the welfare of sows during this period of extremely complex behaviour patterns (Phillips et al., 1992). Crates have been shown to increase posture changing and decrease the time spent standing in the pre-parturient period compared to straw-bedded pens (Cronin et al., 1994, Lawrence et al., 1994). An increase in floor and fixture directed behaviours was also seen in crated gilts (Lawrence et al., 1994) suggesting that the restriction of the environment and lack of substrates leads to the redirection of nestbuilding behaviour. Parturient pigs kept in farrowing crates are not routinely provided with straw or other nesting materials. Baxter (1982) suggested that motivation to build a nest is reduced in the presence of soft materials which would allow udder comfort. However a study by Arey et al. (1991) showed that penned sows continued to perform nest-building behaviour when a pre-formed nest was available suggesting that sows are motivated to perform nest-building behaviours and not simply to produce a suitable nest. In apparent contrast to this finding, a study by Hutson (1988) suggested that sows were not highly motivated to obtain straw during the pre-parturient phase, however the sows used in this study had no prior experience of straw. The provision of sawdust to crated pre-parturient sows has been shown to increase activity and reduce postures that may be indicative of motivational conflict such as sitting (Cronin et al., 1993). The attempted nest-building seen in crated pre-parturient pigs (Jones, 1966a) can therefore be modified and studies have

shown that the provision of straw and covering increases nesting activity and has a subsequent advantageous effect on piglet survival (Cronin and van Amerongen, 1991).

Sows are in lateral recumbency (Jones, 1966b, Randall, 1972) for the majority of the expulsive phase. The time taken to expel all piglets has been reported to be around two hours (Lawrence et al., 1995) however can take up to 16 hours (Signoret, 1975). Blackshaw et al., (1994) found no difference between crated and penned sows in their level of activity during the first day following farrowing. This might suggest that the level of restriction caused by the crating of sows is reduced once farrowing has begun as the motivation to be active is reduced. However sows are normally housed in this restrictive environment during the lactation period until the piglets are weaned at around 21-28 days (English et al., 1982).

1.3 Physiology of Late Pregnancy

It is, as yet, unclear what initiates parturition in the pig. In sheep the role of the foetus in the onset of parturition is identified as removal of the foetal pituitary results in prolonged gestation unless ACTH (adrenocorticotropic hormone) or corticosteroids are administered (Liggins et al., 1969). This suggests that parturition in the sheep is triggered by a rise in foetal adrenal cortisol (Boer et al., 1979). A study by Randell et al., 1990, showed that infusion of piglets with ACTH *in utero* does not alter the length of gestation suggesting that foetal adrenal cortisol is not involved in the initiation of parturition in the pig. However, research carried out on pigs has resulted in a

comprehensive profile of hormonal changes around the onset of parturition.

Similar to other species progesterone, released by the corpora lutea, is required to maintain pregnancy in the pig as at least 1-3 corpora lutea must be present to prevent abortion (Thomford et al., 1984). Between approximately 24 and 10 hours before parturition prostaglandins, which are biologically active phospholipids synthesised in the uterus (Brinsmead et al., 1985), cause luteolysis of the corpora lutea. As the corpora lutea are the main source of progesterone during pregnancy, luteolysis of these structures results in decreased levels of progesterone at this time (Molokwu and Wagner, 1973, Robertson and King, 1974, Ellendorf et al., 1979, Taverne et al., 1979, Taverne et al., 1982, King and Wathes, 1989).

Luteolysis of the corpora lutea results in elevated levels of plasma relaxin (Sherwood et al., 1975, Sherwood et al., 1979, Sherwood et al, 1981, Watts et al., 1988) as this hormone is stored in granules within the large luteal cells of the corpora lutea (Sherwood et al, 1975). Relaxin has been shown to inhibit myometrial activity (Watts et al., 1988) and reduce intra mammary pressure following electrical stimulation of the posterior pituitary in anaesthetised rats (O'Byrne et al., 1986). Relaxin appears therefore to allow a period of preparation of the uterus before the onset of parturition (Taverne et al., 1992). This period of myometrial quiescence allows an increase in oestrogen receptor density as progesterone is known to inhibit this (Hsueh et al., 1975). Oestrogen, which is produced by the placenta through the conversion of cholesterol in the fetal liver (Brinsmead et al., 1985), has been shown to increase in the pig at this time (Robertson and King, 1974, Robertson et al., 1985, McLean et al.,

in press). Oestrogen subsequently facilitates the increase in the number of oxytocin receptors in the uterus and enhances conductivity of the uterine muscle in preparation for labour (Soloff, 1979).

Prior to the onset of parturition elevated levels of plasma oxytocin are required to facilitate uterine contractility (Taverne et al., 1979) with peak levels occurring at the onset of parturition and during the expulsive phase (rat: Higuchi et al., 1985, pig: Lawrence et al., 1995). Relaxin also inhibits oxytocin release perhaps to prevent premature release of oxytocin stored in pituicytes (O'Byrne et al., 1986). Radioactive labelled relaxin has been detected in the rat pituitary suggesting its potential involvement in oxytocin inhibition (O'Byrne et al., 1986). Naloxone, an opioid antagonist, has been shown to reverse this relaxin-induced inhibition of oxytocin release suggesting that this inhibition is mediated via an opioid pathway. Recent work, however, suggests that relaxin may in fact stimulate oxytocin secretion but release is inhibited by terminal actions of an opioid substance, met-enkephalin, which is synthesised within the oxytocin cells (Russell, personal communication). Desensitisation of the terminals may occur over prolonged exposure to this opioid, resulting in a high level of oxytocin release which is required for parturition. Effects of relaxin on the progress of parturition have been shown by infusion of relaxin resulting in a significant increase in the birth interval of infused rats compared to controls with this increase being naloxone-reversible (Jones and Summerlee, 1986). The evidence therefore indicates that relaxin not only inhibits myometrial activity, but also oxytocin release via an opioid pathway which may be relevant to the onset of parturition in the pig.

1.4 Pain at parturition

Giving birth, from human experience, is reported to be an extremely painful process (Melzack, 1992) and research has resulted in numerous different analgesic and anaesthetic drugs administered via various routes to alleviate pain (Brownridge, 1991). The pain experienced by non-human animals during parturition has received little scientific interest, however the research carried out on non-human animals has provided information about the anatomy and neural input to the uterus and cervix.

Studies of the intrinsic nerve supply of the uterus indicates that sensory fibres are more numerous in the cervix and the lower uterine segment than the main body of the uterus (Bonica, 1986). Neural supply to the uterus and cervix is mainly via the hypogastric and pelvic nerves which enter the spinal cord through dorsal roots predominantly in the lumbar region (Steinman et al., 1992, Berkley et al., 1993).

Labour pain is initiated in the uterus due to dilation of the cervix and contraction of the lower uterine segment and there is a correlation between the degree of dilation of these structures to the intensity of pain experienced by humans (Bonica, 1986). There is also a correlation between the onset of uterine contractions and the onset of pain (Corli et al., 1986). This pain results from damaged cells such as those in the uterus producing chemicals, e.g. Substance P, which stimulate chemoreceptive nociceptors, sending messages along Aδ and C fibres (Ochs, 1984) to the spinal cord. Nociceptive information is then transmitted via ascending pathways to higher centres perhaps by Substance P containing neurones (Basbaum and Fields, 1984).

Endogenous opioids are released in response to nociception, and have potent analgesic properties (Dalayeun et al., 1993). Neurones containing opioids such as β-endorphin are thought to control descending pathways at the level of the periaqueductal grey of the brainstem (Basbaum and Fields, 1984) thereby inducing analgesia. Opioids may also have a more local effect at the level of the spinal cord and elevated levels of Dynorphin A (1-17), an opioid which preferentially binds to kappa receptors, have been found in the lumbar region on both Day 22 of pregnancy and during parturition in the rat (Medina et al., 1993a), with levels unchanged in the cervical and thoracic areas of the spinal cord. As the lumbar region is the area of the spinal cord into which the hypogastric and pelvic nerves enter, this opioid may be involved in inducing analgesia around parturition.

Studies have shown that an endogenous opioid-mediated analgesic system exists in parturient rats (Gintzler, 1980, Sander and Gintzler, 1987) and subsequently shown to have a spinal component (Sander and Gintzler, 1987). Hypogastric neurectomy results in reduced analgesia suggesting that stimulation from the lower uterine segment and cervix induces this analgesic system (Gintzler et al., 1983). This pregnancy-induced analgesia has since been shown to exist in parturient women (Cogan and Spinatto, 1986, Whipple et al., 1990).

Opioid-mediated analgesia at parturition may act as a defence against the pain of labour but increased release of opioids in response to nociception may also interfere with parturition and maternal behaviour by the inhibition of oxytocin (Lawrence et al., 1992). This is particularly applicable to domesticated animals as the increase in the

size of offspring may cause increased release of opioids in response to nociception and thus compromise the welfare of the animal.

1.5 Opioid Inhibition of Oxytocin

Opioids, which are endogenous morphine-like substances, are known to inhibit oxytocin release from the neurohypophysis in the rat (Bicknell and Leng, 1982). Endogenous opioids are known to be released in response to stress (Dalayeun et al., 1993), and acute stress during parturition has been shown to decrease plasma oxytocin and prolong parturition in the rat (Leng et al., 1988) and pig (Lawrence et al., 1992).

Opiate receptors in the neural lobe of the pituitary are exclusively κ (Herkenham et al., 1986), and dynorphin, which is co-released with vasopressin (Watson et al., 1982), was thought to be involved by cross-inhibition as dynorphin binds to neural lobe κ receptors (Summy-Long, 1989). However, although dynorphin antagonists have been shown to increase plasma oxytocin in rats, this was under conditions of dehydration in which vasopressin release would be enhanced (Summy-Long, 1989). The role of dynorphin in oxytocin inhibition is questioned further due to work in the rat involving treatment with cholecystokinin (CCK). CCK activates oxytocin release without the release of vasopressin, and when administration was followed by naloxone injection, oxytocin levels increased (Leng et al., 1992). This suggests auto-inhibition as opioid inhibition of oxytocin continues to occur without the activation of the vasopressin system. To ensure that this effect occurred at the pituitary level, electrophysiological

studies involving CCK and naloxone were carried out. CCK did, as expected, increase the firing rate of oxytocin neurones, however this was not potentiated by the administration of naloxone (Leng et al., 1992). The authors suggested a role for extended forms of met-enkephalin, which bind to κ receptors, and are present within oxytocin neurones (Martin and Voigt, 1981). Further evidence for this is that the content of met-enkephalin significantly falls within the neural lobe on Day 21 of pregnancy in the rat suggesting release at this time (Douglas et al., 1993a).

Oxytocin is involved in uterine contractions and expulsion of foetuses, thus a surge of oxytocin is seen at the onset of parturition in sows (Lawrence et al., 1995). This may be due to prolonged exposure to opioid inhibition resulting in desensitisation of the oxytocin neurones. This was also suggested to be the case in rats in which isolated neural lobes were stimulated at the end of pregnancy, and the effect of U50 488 (a κ agonist) was reduced resulting in oxytocin release (Douglas et al, 1993b). The role of endogenous opioids may therefore be to delay the onset of parturition, and the subsequent desensitisation of oxytocin neurones may be involved in the initiation of parturition. As the sow gives birth to many piglets opioids may be involved in spacing the delivery of piglets by preventing the full release of all stored oxytocin from pituicytes. Work by Pumford et al., (1991) has shown that the electrical activity of oxytocin neurones is suppressed following even low doses of morphine suggesting opioid involvement in the control of oxytocin release.

Oxytocin is involved in the onset and maintenance of maternal behaviour in the rat (Pedersen and Prange, 1979, Pedersen et al., 1982, Fahrback et al., 1985) and the

sheep (Keverne and Kendrick, 1992). The administration of morphine sulphate (Rubin and Bridges, 1984, Bridges and Grimm, 1982) and β-endorphin (Mann and Bridges, 1992) into the pre-optic area of the rat disrupts ongoing full maternal behaviour and reduces pup retrieval. These studies therefore suggest that opioid-induced reduction of maternal behaviour is achieved potentially through the inhibitory action of opioids on oxytocin release.

In litter bearing animals, such as the pig, the inhibition of oxytocin by opioids around parturition may be in place to control the initiation of parturition and the delivery of subsequent piglets. However, there may be potential for environmental factors to increase levels of opioids and subsequently prolong parturition and reduce maternal care.

1.6 Stress at parturition

1.6.1 The Stress Response

A stimulus which is perceived to cause potential damage to the biological balance of an animal may be classified as a stressor (Janssens, 1994). The perception of such a stimulus causes signals from the limbic system to be relayed to the hypothalamus resulting in the release of corticotropin releasing hormone (CRH) (Janssens, 1994). CRH is released from nerve terminals into the portal system to cause the subsequent release of adrenocorticotropin hormone (ACTH) from the anterior pituitary (Rivier and Vale, 1985). ACTH which is released into the circulation causes the release of glucocorticoids, such as cortisol, from the adrenal cortex. Glucocorticoids stimulate

catabolic processes to increase blood glucose. This occurs through gluconeogenesis, with lypolysis and the breakdown of proteins resulting in free-fatty acids and amino acids for subsequent gluconeogenesis (Munck et al., 1984). A schematic representation of the hypothalamic-pituitary-adrenal (HPA) axis based on Janssens, (1994) can be seen in Figure 1.

Hypersecretion of glucocorticoids is potentially harmful to an animal due to possible immunosuppression (Baxter and Tyrell, 1987) and a negative feedback mechanism must therefore be in place to prevent this. As can been seen from Figure 1, cortisol negatively feedbacks to inhibit further release of ACTH by acting at the pituitary, hypothalamus and the CNS (Keller-Wood and Dallman, 1984).

Chronic stress has been shown to increase the responsiveness of the adrenal gland in the rat (Sakellaris and Verniknos-Danellis, 1975) and more recently the responsiveness of the adrenal gland in pigs exposed to tethering has been shown to increase (Janssens, 1994). This suggests that during chronic stress there is a decreased sensitivity of the glucocorticoid feedback system (Akana et al., 1992). Therefore restrictive environments such as the farrowing crate may have the potential to alter adrenal sensitivity as a result of chronic stress and lead to problems such as immunosuppression.

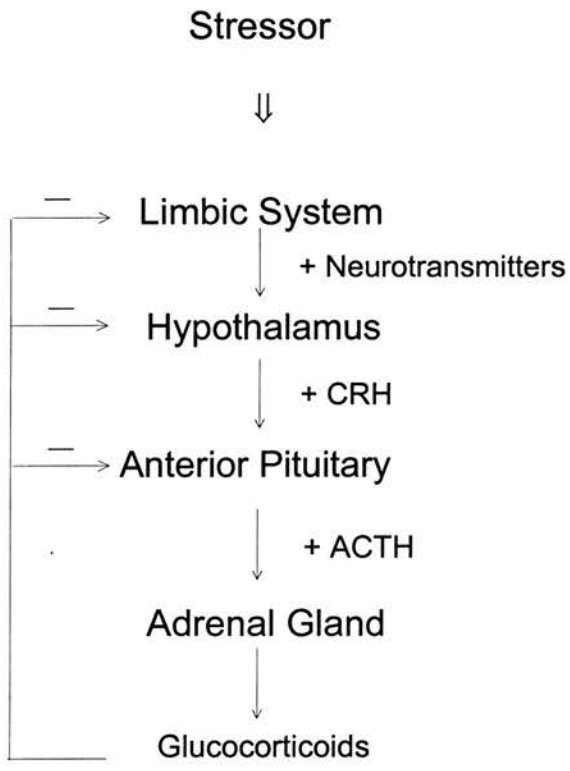


Figure 1 - Diagrammatic representation of HPA axis. A stressor results in signals from the limbic system to the hypothalamus, which causes CRH to be released into the portal system. CRH subsequently causes the release of ACTH into the circulation from the anterior pituitary. Plasma ACTH induces release of glucocorticoids into the periphery (Janssens, 1994). +; stimulatory, -; inhibitory

1.6.2. Effect of Parturition on the Stress Response

Parturition in itself can be an extremely stressful process and work has shown an increase in cortisol (Kaupilla et al., 1974) and ACTH during parturition in women (Kaupilla et al., 1974, Fettes et al., 1984, Fajardo et al, 1994). These blood parameters appear to be indicative of anxiety as a significant correlation between plasma cortisol and anxiety scores was found in women during labour (Lederman et al., 1978). Studies examining blood parameters indicative of physiological stress in pigs have also found an increase in plasma cortisol particularly in the phase prior to expulsion (Killian et al., 1973, Meunier-Salaun et al., 1991). A more detailed study of changes in plasma cortisol during parturition found that cortisol levels continued to increase during the expulsion of the numerous piglets (Lawrence et al., 1994). The authors suggested that this may be a response to factors specific to parturition such as pain or novelty. Uterine contractions and piglet expulsion could be potential stress-inducing factors due to the associated pain and novelty. In addition, the piglets themselves are effectively novel objects and a study by Hutson et al., (1992) found that the reaction time of gilts to distress calls of piglets was shorter than that for older sows suggesting an element of novelty. Parturition in the pig therefore may be associated with 'stress' resulting from certain aspects of the parturition process itself.

1.6.3. Environmental Effects on the Stress Response

There are relatively few studies comparing the effect of different environments on physiological responses in the pig. Work by Janssens (1994) has shown that tethering during the oestrus cycle in pigs results in elevated daily mean plasma cortisol

concentrations compared to loose housed pigs demonstrating environment induced chronic stress.

When we consider the very complex behaviour patterns which are performed during the pre-farrowing phase it may be suggested that restrictive environments may have a particularly adverse effect during the pre-farrowing period. Work by Cronin et al., (1991) has shown that introduction into a crate 10 days pre-farrowing caused elevated plasma cortisol compared to pigs introduced to a pen. This may be accounted for by the restriction of the environment or the novelty associated with such an environment. During the pre-farrowing nest-building period elevated levels of plasma cortisol have been seen in crated gilts in comparison to gilts housed in straw-bedded pens suggesting that the restriction of their behaviour at this time causes physiological stress (Lawrence et al., 1994). In the same study there were no difference in plasma cortisol between the gilts in the two environments during the expulsive phase. This may be, as mentioned, due to the inactivity of the gilts at this time reducing the restrictive effect of the crate. In addition work by Gilbert et al., (1997) has shown that restricting space allowance during farrowing does not increase plasma cortisol concentrations. Pigs are normally housed in crates throughout lactation which is around 28 days (English et al., 1982) and evidence from a study by Cronin et al., 1991 indicates chronic stress in crated gilts at the end of the lactation period due to elevated levels of plasma cortisol on Day 28 of lactation (before weaning).

Overall the introduction to crates appears to cause physiological stress in pigs, however the animals appear to adapt to the environment before the onset of nest-

building. Elevated levels of plasma cortisol have been found during the nest-building phase suggesting that this may be a period in which the crate compromises the welfare of the pig. Throughout the expulsive phase the lack of environmental effects on plasma cortisol may be due to the inactivity seen at this time. Crating during lactation may have the potential to reduce the welfare of the pig. All of these stages around farrowing need to be investigated in more detail to determine the impact of crating on the welfare of farrowing and lactating pigs.

Obviously this type of research requires a huge amount of technical input with regard to the collection of data, therefore a technical team, which has been duly acknowledged, was allocated to help with this work. The thesis contains five experimental chapters resulting from three experiments. The first experiment, which is of my own design and which I had a large input into collection of data, is described in chapters two and three. The collection of blood samples for the experiment described in chapters four and five was carried out simultaneously with the first experiment, and all the radioimmunoassays and subsequent analyses were carried out by myself. I was responsible for the design, data collection, radioimmunoassays and data analysis of the final experiment which is described in Chapter 6.

Aims of the thesis

1. To determine if pregnancy-induced analgesia exists in the pig, and if so whether this is mediated via endogenous opioids. The effect of these endogenous opioids, perhaps through the inhibition of oxytocin, on maternal behaviour will also be examined.

2. Farrowing crates are widely used, and the effect of this restrictive environment on the stress physiology and behaviour of the pig will be examined in the pre-parturient period, a time of increased activity due to nest-building. During the expulsive phase itself the stress physiology of the pig will be examined in relation to restriction and opioid mediation.

3. Stress during the expulsive phase may be due to a number of factors and this study aims to determine the effect of piglet expulsion on the stress physiology of pigs housed in farrowing crates and straw-bedded pens.

Overall the thesis aims to better understand the physiology and behaviour of the pig around parturition, and to determine whether there are adverse effects of environmental restriction in terms of physiological stress and potential implications for maternal behaviour.

Chapter 2

Opioid-mediated changes in nociceptive threshold during pregnancy and parturition in the sow

2.1 *Summary*

This study aimed to investigate if pregnancy-induced hypoalgesia occurs in the sow, and to examine the role of endogenous opioids which are known to be released in response to nociception. Sixteen Large White x Landrace multiparous sows were tested in straw bedded pens (2.5m x 2.5m) during weeks 4, 8 and 12 of pregnancy and over the farrowing period. Testing involved thermal stimulation of 8 areas on the rear-quarters of the sows with a CO_2 infra-red laser until a physical response was seen (tail flick, leg move or muscle twitch) or for a maximum of 16 seconds. Over the farrowing period testing was more frequent, and at 3¾ hours after the birth of the first piglet, half the sows received an injection (i.m.) of an opioid antagonist naloxone (N) (1mg kg^{-1} body weight) with the remainder receiving a control dose of saline (S). Responses were recorded 15 and 30 minutes post-injection. There was no significant difference between response times over weeks 4, 8 and 12 of pregnancy ($p=0.152$), however a significant rise was seen from week 12 to 5 days before parturition ($p=0.002$). Response times continued to rise until the birth of the first piglet by which time the majority of sows had stopped responding within 16 seconds ($p<0.001$). Response times fell over days 1, 2 and 7 post-partum. After administration of naloxone response times fell compared to control animals at 15 mins ($p<0.001$) and 30 mins ($p<0.01$) post-injection. These results suggest that nociceptive threshold increases during late pregnancy in the sow, perhaps as an endogenous defence against labour pain, and that during parturition this change in nociceptive threshold is, at least in part, opioid-mediated. Oxytocin is known to be inhibited by endogenous opioids at parturition, thus future research should consider the potential role of increased nociception at birth as a negative feedback to oxytocin release.

2.2. Introduction

The human birth process is associated with increased nociception and has consequently received a great deal of scientific interest. However there has been very little interest in the pain experienced by farm animals during parturition. With the genetic selection for faster growth leading to increased size of offspring, there are potential welfare implications for the mother, both in terms of the progress of parturition and the onset of maternal behaviour.

An increase in nociceptive threshold has been shown in both women (Cogan and Spinnato, 1986, Whipple et al., 1990) and in the rat (Gintzler, 1980) perhaps as an endogenous defence against the pain of parturition. Whipple et al. (1990) showed that tactile threshold did not change over the parturient period indicating that the changes seen in nociceptive threshold were not due merely to distraction caused by the labour process itself. Endogenous opioids are known to be released in response to nociception, and have potent analgesic properties (Dalayeun et al., 1993), and Gintzler (1980) suggested that the pregnancy-induced hypoalgesia observed in the rat was mediated via endogenous opioids as the rise in nociceptive threshold did not occur in rats administered (s.c.) with naltrexone, an opioid antagonist. A further study in rats suggested that a spinal opioid mechanism was involved as a significant decrease in nociceptive threshold was seen when naltrexone was administered intrathecally (Sander and Gintzler, 1987).

Neural supply to the uterus and cervix is mainly via the hypogastric and pelvic nerves which enter the spinal cord through dorsal roots predominantly in the lumbar region (Steinman et al., 1992, Berkley et al., 1993), and therefore this area may be particularly involved in the hypoalgesia associated with late pregnancy. Transection of the hypogastric nerve significantly reduced the increase in nociceptive threshold in rats over the pre-parturient period compared to sham operated animals (Gintzler et al., 1983). Pelvic neurectomy in rats also causes attenuation of the rise in nociceptive

threshold associated with uterocervical mechanostimulation (Gintzler and Komisaruk, 1991). Elevated levels of Dynorphin A (1-17), an opioid which preferentially binds to kappa receptors, were found in the lumbar region on both Day 22 of pregnancy and during parturition in the rat (Medina et al., 1993a), with levels unchanged in the cervical and thoracic areas of the spinal cord. In addition intrathecal administration of a kappa specific antagonist, nor-binaltorphimine, has been shown to reduce nociceptive threshold in rats on Day 20 of pregnancy (Sander et al., 1988). Thus it is likely that uterine distension and cervical stretching, which occur during the later stages of pregnancy and parturition, are involved in activation of pregnancy-induced hypoalgesia by stimulation of afferents in the hypogastric and pelvic nerves. It also appears that this pregnancy-induced hypoalgesia is mediated via a spinal κ-opioid mechanism. However further work in the rat has suggested that simulation of pregnancy by administration of 17β-estradiol and progesterone modulates an opioid analgesic system (Dawson-Basoa and Gintzler, 1993), and also increases spinal cord Dynorphin A (1-17) in the lumbar region of the spinal cord (Medina et al., 1993b). Therefore, both hormonal and neuronal factors may be involved in the activation of pregnancy-induced hypoalgesia.

It has been shown initially in the rat (Leng et al., 1988) and then in the pig (Lawrence et al., 1992) that environmental stress inhibits oxytocin and slows delivery and that both of these effects are naloxone-reversible. Thus opioids released in response to increased nociception at parturition, perhaps due to an increase in the size of offspring, may also act to inhibit oxytocin and may compromise the welfare of the sow by prolonging parturition and interfering with maternal behaviour. Therefore this study aimed to initially determine whether changes in nociceptive threshold occurred over pregnancy and parturition in the sow, indicative of pregnancy-induced hypoalgesia. Secondly, we wished to demonstrate whether any changes were mediated via an opioid mechanism, with a view to further research considering the potential for increased nociception at parturition to modify oxytocin release.

2.3. Animals, materials and methods

2.3.1. Animals and Housing

The experimental protocol was reviewed and approved by the Animal Experiments Committee of the Scottish Agricultural College, Edinburgh, Scotland, and the procedures used were in accordance with the Animals (Scientific Procedures) Act (1986).

The subjects of this study were 16 Large White X Landrace sows (Easter Howgate Pig Unit, Milton Bridge, Penicuik, Midlothian - mean parity ± s.e.= 3.7 ± 0.04) studied in 2 replicates of 7 and 9 sows respectively. The sows were initially group housed in straw-bedded pens (2.6m x 4.1m) and fed 2.5 kg day^{-1} of a commercial diet providing 13 MJ DE kg^{-1} at 0800hrs. A boar was introduced daily and was used to serve the sows on two consecutive days. The expected parturition day (EPD) was calculated as 114 days (16.3 weeks) after the first service. Once pregnancy was confirmed at around 32 days after service, the sows were housed in groups of 3 or 4 in a semi-open building in a pen consisting of a concrete yard (6.0 m x 4.0 m) with a straw bedded kennel area at the back (6.0 m x 1.5 m). The sows were floor fed 2.5 kg day^{-1} of the same commercial feed at 0800hrs. Artificial lighting was provided between 0730 and 1700hrs.

2.3.2. Experimental Housing

For testing, sows were moved with their group to a temperature controlled room (18° C) for approximately one week. This occurred at weeks 4, 8 and 12 of pregnancy (Section *2.3.1*), with artificial lighting provided as before. Each sow was moved to an individual straw bedded pen (2.5 m x 2.5 m) and trough fed the same feed at the previous amount and time. These pens were also used for the sows to farrow (give birth), at which time a creep (heated area for piglets) was added (Section *2.3.2*). Over the farrowing period the feed was changed to a commercial diet providing 13.75 MJ DE kg^{-1} and containing 18% protein. This lactation diet was offered in 2 meals at

0800 and 1600hrs. The level of feed increased from 2.5 kg day^{-1} depending on time since farrowing.

2.3.3. Measurement of Nociceptive Threshold

The noxious stimulus applied in this study to measure nociceptive threshold was a thermal stimulus produced by a CO_2 infra-red laser (MPB Technologies, Dorval, Quebec). The power setting decided upon (1.5W) was that which produced a mean skin temperature of 52.3°C ± 0.76 (s.e.m). In a pilot study using non-test sows this setting consistently gave rise to behavioural responses at around 8 to 10 seconds, however after 15 seconds skin damage occurred. Therefore 15 seconds was used as the end point. The specific areas on the rear end of the sows which were to be stimulated with the laser were also decided upon within this pilot study (Figure 1). The areas were shaved and marked clearly with a permanent pen, and this pattern of areas was used for all sows throughout the experiment. Within one test the 8 areas were stimulated at random. The heat producing beam worked in conjunction with a timer, such that when the observer started the timer the heat producing beam came on automatically. When a physical response was observed (tail flick, movement of the back leg, muscle twitch), the timer was stopped and the time and nature of the response was recorded. If no response was observed within the 15 seconds, the laser automatically stopped, and a value of 16 seconds was allocated for statistical purposes.

The surface temperature of each area was recorded using a remote infra-red thermometer (Cyclops Compac 3, Minolta/Land, Sheffield. England) before stimulation with the laser to determine the effect of initial skin temperature on the response time of the pig. All reflective objects were removed from the room and all safety procedures were employed in accordance with the University of Edinburgh Radiation Protection Committee (The University of Edinburgh, 1993).

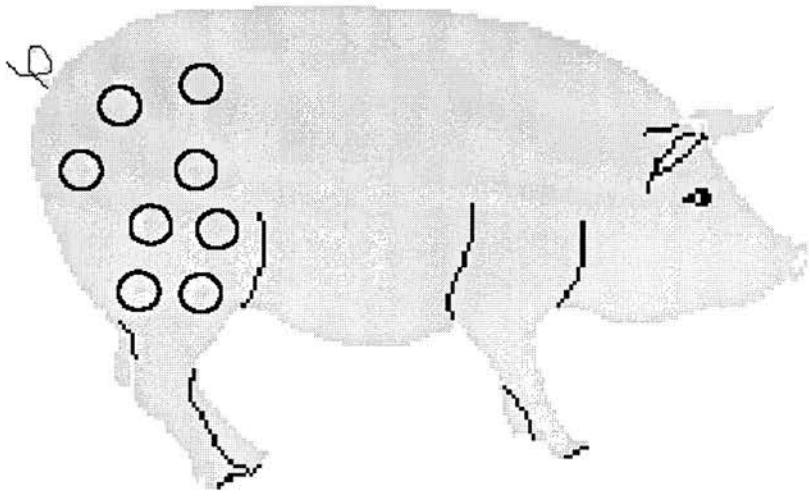


Figure 1 - Pattern of areas used on all sows (n=16) over the entire study.

2.3.4. Heart Rate Measurements

A further pilot study on non-test sows was carried out to measure heart rate over the 15 second stimulation to determine any stress-inducing effects of the test. This involved using a heart rate monitor (Polar Electro PE3000, Kempele, Finland) which was attached to the pigs during the 15 second laser stimulation. The heart rate was measured just prior to each stimulation, and then 5, 10 and 15 seconds during the stimulation. The data were analysed using a repeated measures analysis of variance.

2.3.5. Pregnancy up to Week 12

After sows had been moved to the test pens at weeks 4, 8 and 12 of pregnancy, they were allowed a 2 day habituation period. Following this period the laser tests were carried out on 3 alternate days in week 4, and on 2 alternate days in weeks 8 and 12. On each test day, one side of the pig was tested in the morning and the other side in the afternoon. The sides were tested in the opposite order on the following test day. The sows were only tested when they were in a lying position and were awake. All laser sessions were recorded on video (Panasonic 24 hour time lapse video recorder, AG - 6024)

2.3.6. Parturition

The sows were moved into the test pens 7 days before the EPD. Tests were carried out, again in the morning and afternoon, on day -5 (5 days before EPD) and continued on consecutive days until day -3. On day -2 the morning and afternoon tests were carried out, and the sows were also tested at midnight. They were then tested every 8 hours from this point until nest-building began, at which point tests were carried out every 4 hours until the delivery of the 1st piglet. The indicators used to determine the onset of nest-building were oral manipulation of straw, bars and the trough. If nest-building began before midnight on day -2, 4 hourly tests were started from the onset of nest-building. A laser test was carried out following the birth of the first piglet (mean ± s.e. = 13.43 ± 5.1 minutes post-piglet 1). Testing was also carried out 2 and 6 hours after the 1st piglet was born. Alternating sides for testing was difficult at this time due to the sows tending to lie on the same side during parturition, therefore tests were carried out on the exposed side. Morning and afternoon tests were resumed on days 1, 2 and 7 post-partum. All laser tests were recorded on video, and piglet weight, sex and time of birth were recorded. Responses to stimulation were measured on six of the sixteen sows post-weaning, which was one day after the piglets had been removed following a 28 day lactation period.

2.3.7. Effect of Naloxone

To test the involvement of opioids, naloxone, an opioid antagonist, was administered (1mg kg^{-1} body weight) intramuscularly in the neck to half of the sows at 3¾ hours after the birth of the 1st piglet, and a laser test carried out 15 and 30 minutes post-injection (4 and 4¼ hours after the 1st piglet). The other half of the sows received saline as a control and were tested in the same way post-injection.

2.3.8. Observers

Five observers were required to conduct this study to allow night work to be carried out. The experiment was, as mentioned earlier, carried out in 2 replicates, therefore reducing the possibility that changes in response times were due to the improved definition of responses by observers. Also within each replicate the sows EPDs were staggered over time so that pregnancy and parturition measurements were being carried out simultaneously.

2.3.9. Statistical Analysis

2.3.9.1 Pregnancy

The data over pregnancy were normally distributed and were analysed by a repeated measures analysis of variance (Genstat, Version 5, Lawes Agricultural Trust, Rothamsted Experimental Station) using a blocked structure for replicate, pig, week and time of day. Factors used were week (4, 8, 12), time of day (am, pm), area (8 levels), replicate (2 levels) and parity (6 levels). The effect of the observer could not be added into the blocking structure as it was unbalanced, therefore a regression analysis (Minitab, version 7.1) was performed between observer and response time to determine any effect. A second regression analysis was performed between initial skin temperature and response time.

2.3.9.2. Parturition

The data obtained over the parturition period were not normally distributed due to a large proportion of the observations being at the 16 second cut-off point, and therefore non-parametric statistics were applied. Initial skin temperature and observer were not accounted for over this period as analysis of the pregnancy data showed that neither of these variables affected response time. The data were divided into responses at specific times or responses pooled over time periods (Table I). In order to analyse temporal changes in nociceptive threshold the difference between two successive times was calculated and a Wilcoxon Rank Sign Test (Minitab, version 7.1) used to determine whether the change was significantly less or greater than zero.

2.3.9.3. Pregnancy and Parturition

A Wilcoxon Rank Sign Test (Minitab, version 7.1) was used to determine changes in response time between week 12 of pregnancy and 5 days pre-parturition. The post-weaning values recorded for six sows were compared, using a paired t-test, with the mean values over pregnancy for the six sows.

2.3.9.4. Effect of Naloxone

A Mann Whitney Test (Minitab, version 7.1) was used to compare response times of sows receiving naloxone and those receiving saline at both 15 and 30 minutes post-injection.

Table I - Description of pooled time periods and specific time points around parturition used in the statistical analysis

Time		Description
Pooled Periods (frequency of tests)	Specific Times	
Day -5 (twice daily)		-5 days
Day -4 (twice daily)		-4 days
Day -3 (twice daily)		-3 days
-48 to -24 hours (8 hourly)		-48 hours
-24 to -12 hours (8 hourly)		-24 hours
-12 hours to the birth of piglet 1 (4 hourly)		-12 hours
	Birth of piglet 1 (P)	0
	P + 2 hours	+2 hours
	P + 6 hours	+6 hours
	P + 12 hours	+12 hours
Day +1 (twice daily)		+1 day
Day +2 (twice daily)		+2 days
Day +7 (twice daily)		+7 days
Post-weaning (twice daily)		pw

2.4 Results

2.4.1. Length of parturition, litter size and weight

The mean duration of parturition was 3.99 ± 0.67 (s.e.) hours, with the mean inter-piglet birth interval being 23.77 ± 3.70 (s.e.) minutes. The effect of naloxone on these variables could not be determined as most sows had had their piglets by the time of the injection. The mean litter size was 11.13 ± 0.74 (s.e.) and the mean weight of the piglets was 1.59 ± 0.08 (s.e.) kg.

2.4.2. Heart Rate Measurements

There was no effect of laser stimulation on the heart rate of the non-test sows in the pilot study. The mean heart rates (± s.e.) during the laser stimulation were 85.04 (± 1.42), 85.05 ± (1.38), 85.44 ± (1.37) and 85.37 ± (1.43) for 0, 5, 10 and 15 seconds respectively. This suggests that a rise in response time is not due to any stress-inducing effects of the test.

2.4.3. Pregnancy

Response times did not change significantly during early and mid pregnancy (mean response time (s) ± s.e = 8.26 ± 0.56 for week 4, 10.04 ± 0.63 for week 8 and 9.45 ± 0.58 for week 12).

A regression of the initial skin temperature of each area on response time showed that temperature accounted for less than 1% of the variation in response time (R-sq = 0.6%, t = 0.3). Observer also explained less than 1% of the variation in response time (R-sq = 0.8%, t = -3.72).

2.4.4. Parturition

As can be seen in Figure 2, there was an increase in response time between week 12 of pregnancy and -5 days ($W=102$, $p<0.01$). A significant rise in response time can be seen between -3 days and -48 hours ($W=117$, $p<0.05$), and also between -24 hours and -12 hours ($W=101$, $p<0.05$) (Figure 2). At the birth of the first piglet, a highly

significant increase in response time had occurred relative to -12 hours ($W=136$, $p<0.001$). The mean response time had reached the maximum value of 16 seconds (no response to the laser) for most sows and remained at this level until +2 hours. A slight decrease in response time was found between +2 hours and +6 hours ($W=3.0$, $p<0.05$). A gradual decline in response time was seen over +1 day, +2 days and +7 days. By the post-weaning period, the response times of the six sows tested did not differ significantly from +7 days. Also the response times of the six sows at post-weaning were significantly higher ($T=2.92$, $p<0.05$) than their response times during pregnancy.

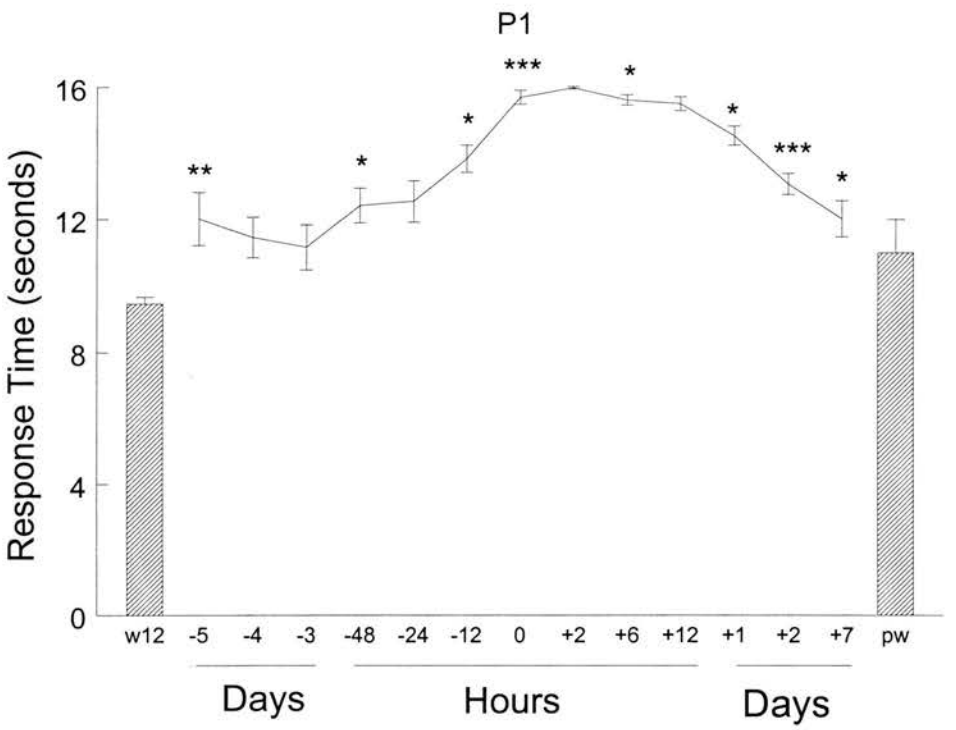


Figure 2 - Mean response times (± s.e.) over the farrowing period (n=16). A mean for week 12 (w12) is depicted to indicate levels during pregnancy (n=16). A mean for the post-weaning (pw) measurements (n=6) is also given. Significance levels at a time period indicate whether a rise or fall in response time has occurred from the previous time period. P1=piglet 1. * $p<0.05$, ** $p<0.01$, *** $p<0.001$.

2.4.5. Effect of Naloxone

At 15 minutes after the injection, most sows receiving saline did not respond within the 15 second stimulation (16 seconds allocated), whereas the response times of sows receiving naloxone had fallen significantly ($W=4660.5$, $p<0.001$) (Figure 3). The sows receiving naloxone also had significantly lower response times ($W=4028.5$, $p<0.01$) at 30 minutes post-injection. There was no difference between the response times of the allocated groups at +2 hours (1¾ hours prior to the injection). Again at +6 hours (2¼ hours post-injection) there was no difference between the response times of the allocated groups suggesting no persisting effects of naloxone.

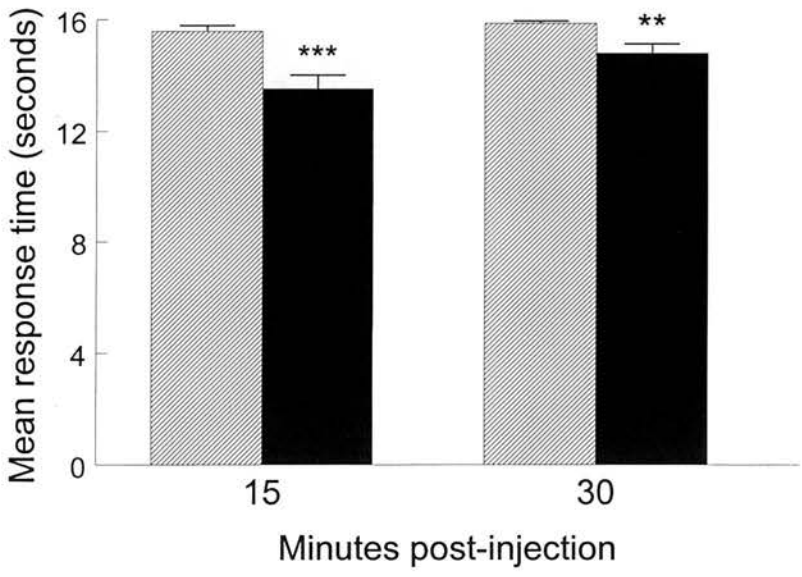


Figure 3 - Mean response times (+ s.e.) at 15 and 30 minutes post-injection of either saline (▨) (n=8) or naloxone (■) (n=8). ** $p<0.01$, *** $p<0.001$.

2.5 Discussion

The results of this study suggest that hypoalgesia occurs during late pregnancy and parturition in the sow. The attenuation of the rise in nociceptive threshold seen by naloxone suggests that an opioid mechanism is partly involved at least during parturition. This provides further evidence for pregnancy-induced hypoalgesia which has previously only been demonstrated in the rat (Gintzler, 1980) and women (Cogan and Spinnato, 1986, Whipple et al., 1990). Response times did not increase as a result of the repeated use of the noxious stimulus, as firstly the response times did not increase over the first 12 weeks of pregnancy, and secondly a decline in response time was observed in the post-partum period.

The pharmacokinetics of naloxone have not been studied in the pig; in sheep however it has been shown to have a half-life of around 40 minutes (Alavi et al., 1994). This short half-life may account for the reduction in significance level between 15 and 30 minutes post-injection. Although the fall in response time seen in the sows receiving naloxone was significant, the reduction appears to be relatively small. This may be partly a result of the cut-off point being set at 15 seconds, as the actual response times of the saline animals may have been considerably longer. Also naloxone did not fully reverse the rise in nociceptive threshold suggesting that opioids are only partly responsible for hypoalgesia during parturition or more simply that the dose and route of administration used in this study was insufficient.

As naloxone was administered intramuscularly in this study, the site of action can not be determined. This is also the case for the work carried out in rats by Gintzler (1980), as naltrexone was administered subcutaneously. However it has been shown that intrathecal administration of naltrexone in pregnant rats causes a decrease in nociceptive threshold suggesting a spinal opioid pathway is involved in pregnancy-induced hypoalgesia (Sander and Gintzler, 1987).

Naloxone exhibits some preference for μ-receptors (Herz and Almeida, 1989), however has been shown to bind to κ-receptors (Herkenham et al., 1986) and δ-receptors (Sharif and Hughes, 1989). Therefore the specific opioid ligand responsible for this pregnancy-induced hypoalgesia in the pig cannot be concluded. Work in rats has shown that nor-binaltorphimine (i.t.), a κ-selective antagonist reduced nociceptive threshold of rats to an electric foot-shock during day 20 of pregnancy (Sander et al., 1988), suggesting the involvement of a κ-opioid system. Dynorphin A (1-17), an opioid which preferentially binds to kappa receptors, has been shown to increase in the lumbar region of the spinal cord during day 22 and parturition in the rat (Medina et al., 1993a). The main afferent nerves of the uterus and cervix, the hypogastric and pelvic nerves, enter the spinal cord through various dorsal roots, but predominantly in the lumbar region where, as mentioned, dynorphin A (1-17) levels increase around parturition. Work involving transection of the hypogastric nerve during pregnancy and parturition (Gintzler et al., 1983), and pelvic neurectomy prior to uterocervical mechanostimulation (Gintzler and Komisaruk, 1991) reduced hypoalgesia. The hypogastric nerve terminal fields are in lamina I and V of the dorsal horn, which is where Dynorphin A (1-17) is predominantly found (Dubner and Hargreaves, 1989), and lamina I is very rich in opiate receptors (Sander and Gintzler, 1987). It is suggested therefore that uterine distension and cervical stretching, which are essential parts of the parturition process, stimulate afferents running in the hypogastric and pelvic nerves and activate a spinal, probably kappa, opioid system.

Results obtained in this study suggest that response times in the sow had increased significantly by at least 5 days pre-parturition i.e. before the onset of uterine contractions and cervical stretching. Other studies indicate that the activation of pregnancy-induced hypoalgesia may also be under endocrinological control. Simulation of pregnancy using 17-β oestradiol and progesterone results in hypoalgesia in rats (Dawson-Basoa and Gintzler, 1993), with nociceptive thresholds showing a similar pattern to that seen in pregnant rats (Gintzler, 1980). Both studies

showed that the increase in nociceptive threshold was naltrexone reversible suggesting involvement of an opioid mechanism. Dynorphin A (1-17) levels increased in the lumbar region of the spinal cord when pregnancy was simulated with 17-β oestradiol and progesterone (Medina et al., 1993b), suggesting that this opioid appears to be involved in pregnancy-induced hypoalgesia. A more recent study has suggested that this simulation of pregnancy does activate spinal κ-opiate receptor analgesic mechanisms (Dawson-Basoa and Gintzler, 1996). Therefore pregnancy induced changes in sex-steroid concentrations may activate pregnancy-induced hypoalgesia, whilst uterine distension and cervical stretching accentuate the hypoalgesia around parturition. A further accentuation of hypoalgesia has also been shown after ingestion of placenta (Kristal et al., 1985) and amniotic fluids (Kristal et al., 1990) in rats. However the placenta was removed in the present study and cannot account for changes in nociceptive threshold.

The response times of the sows at one day after weaning was significantly higher than those recorded during pregnancy, however this may have been due to stress associated with the separation from their piglets. Previous work in rats has shown that nociceptive thresholds are low during lactation but increase following weaning suggesting stress-induced analgesia at this time (Cruz et al., 1996).

Pregnancy-induced hypoalgesia results from, at least in part, an increased level of opioids in response to both hormonal and neuronal factors which occur at this time. The consequence of these opioids may not be solely the modification of the perception of pain. Evidence suggests that opioids inhibit oxytocin release from the neurohypophysis in the rat (Bicknell and Leng, 1982), and both μ and κ opioids are involved in the reduction in firing rate of oxytocin neurones (Douglas et al., 1995). It is not known if the opioid mechanism involved in pregnancy-induced hypoalgesia is restricted to the spinal cord or is also active in the brain. If a brain opioid pathway was involved then this could provide a route for excessive noxious stimulation of the

afferent nerves of the uterus and cervix to affect oxytocin release. For the progress of parturition, oxytocin must be released to allow expulsion of the foetuses, and is also involved in maternal behaviour (Fahrback et al, 1985), and therefore inhibition of oxytocin may compromise the welfare of both the sow and her piglets.

In conclusion, this study provides evidence that, in the sow, an endogenous analgesic mechanism(s) is activated during late pregnancy and parturition. This appears to be mediated, at least in part, via an opioid mechanism during parturition. The effect of the resulting opioid activity as a negative feedback to oxytocin release should be investigated.

Chapter 3

The responsiveness of sows to their piglets in relation to the length of parturition and the involvement of endogenous opioids.

3.1. Abstract

The aim of this study was to describe maternal behaviour in the pig and to investigate the effect of endogenous opioids on maternal responsiveness. The behaviour of 16 Large White X Landrace female pigs was recorded around farrowing which involved recording the pig's posture and her response when piglets were present at her nose. To determine the role of endogenous opioids, sows were injected (i.m.) with either naloxone, an opioid antagonist, (1mg kg^{-1} bodyweight (n=8)) or saline (n=8) at 3¾ hours after the birth of the first piglet. Generally the initial period following the birth of the first piglet seemed to be the most active after which the sows spent almost all of the time in lateral recumbency. The results also show that farrowing sows are generally unresponsive to their piglets during farrowing. Sows receiving naloxone became more responsive towards their piglets. The changes seen in posture and responsiveness to piglets were delayed in sows with a longer parturition suggesting some involvement of cumulative piglet births on passivity. It is proposed that opioid-mediated passivity in the pig, characterised by lateral lying and unresponsiveness to piglets, may be advantageous by maximising suckling opportunities and reducing the risk of crushing piglets and of attracting predators to the nest.

3.2. Introduction

The birth process, from human experience, is associated with pain and stress, however this association in non-human animals has received little consideration. Yet the hypoalgesia which occurs in women around parturition (Cogan and Spinnato, 1986, Whipple et al., 1990), as an endogenous defence against the pain of parturition, also occurs in rats (Cruz et al., 1996, Gintzler, 1980) and the pig (Jarvis et al., 1997a). The increased hypothalamic-pituitary-adrenal (HPA) activity which is seen in the human towards and during parturition (Fettes et al, 1984, Fajardo et al., 1994) is also seen in the pig (Jarvis et al., 1997b, Lawrence et al., 1994) suggesting that parturition causes physiological stress in both these species. The effects of pain and stress on the maternal behaviour of the pig has rarely been considered.

Endogenous opioids, which have morphine-like properties, are released in response to stress and pain (Fields and Basbaum, 1994), and have been shown to disrupt maternal behaviour in the rat. Administration of morphine sulphate (Rubin and Bridges, 1984) and β-endorphin (Mann and Bridges, 1992) into the pre-optic area of rats results in reduced full maternal behaviour and retrieval of pups to the nest. This effect of opioids on maternal behaviour may be through the inhibition of oxytocin both at the level of the hypothalamus (Bicknell, 1985) and the neurohypophysis (Bicknell and Leng, 1982, Leng et al., 1992) as oxytocin is known to be involved in the onset of maternal behaviour in rats (Pedersen and Prange, 1979, Pedersen et al., 1982).

Previous studies have qualitatively described the behaviour of crated (Jones, 1966b, Randall, 1972) and free-ranging (Jensen, 1986) sows during farrowing and found that sows generally lie in lateral recumbency during the majority of farrowing and change posture most frequently following the birth of the first and second piglet. Therefore the objective of this work was to quantify the maternal behaviour of the sow in terms of her activity and responsiveness to her piglets. The extent to which opioids are involved in the behaviour of the sow at this time was also investigated. We

hypothesised that pigs should show an enhancement of maternal responsiveness when subject to opioid antagonism given that opioids inhibit oxytocin during parturition in the rat (Leng et al., 1988) and the pig (Lawrence et al., 1992), and that administration of opioids inhibits maternal behaviour in the rat (Mann and Bridges, 1992, Rubin and Bridges, 1984).

3.3. Animals, materials and methods

3.3.1. Animals and Housing

The subjects of this study were 16 Large White X Landrace sows (mean parity (± s.e.) = 3.7 ± 0.04) studied in 2 replicates of 7 and 9 sows respectively. The sows were initially group housed in straw-bedded pens (2.6m x 4.1m) and fed 2.5 kg day^{-1} of a commercial diet (13 MJ DE kg^{-1}) at 0800. A boar was introduced daily and was used to serve each sow on two consecutive days. The expected parturition day (EPD) was calculated as 114 days after the first service. Once pregnancy was confirmed at around 32 days after service, the sows were housed in groups of 3 or 4 in a semi-open building in a pen consisting of a concrete yard (6.0 m x 4.0 m) with a straw bedded kennel area at the back (6.0 m x 1.5 m). The sows were floor fed 2.5 kg day^{-1} of the same commercial feed at 0800. Artificial lighting was provided between 0730 and 1700.

3.3.2. Experimental Housing

Five days before the EPD each sow was moved to an individual straw bedded pen (2.5 m x 2.5 m) in a temperature controlled room (18°C), and trough fed using the same feeding schedule described previously. After farrowing the feed was changed to a commercial diet providing 13.75 MJ DE kg^{-1} and contained 18% protein. This lactation diet was offered in 2 meals at 0800 and 1600. The level of feed increased from 2.5 kg day^{-1} depending on proximity to farrowing.

3.3.3. Effect of naloxone

At 3¾ hours after the birth of the first piglet (BFP) the sows either received an injection (i.m.) of saline or naloxone, an opioid antagonist, at a dose rate of 1mg kg^{-1} body weight.

3.3.4. Observations Periods

The sows behaviour was recorded on time-lapse video (Panasonic, AG - 6024) beginning 3 days before the EPD. This continued until the birth of the first piglet, when real-time recording began and continued over the six hours following the birth of the first piglet. Time lapse recording was used again over days 1, 2 and 7 post-farrowing.

During specific observation periods the behaviour of the sows was recorded continuously from the video material. Two hours (1030-1130 and 1300-1400) were observed on the two days prior to farrowing to obtain baseline measurements, and on days 1, 2 and 7 post-farrowing. The behaviour of the sows was also recorded during the six hours following the birth of the first piglet and divided into six periods; 0-1, 1-2, 2-3, 3-4, 4-5, 5-6 hours. As naloxone and saline were administered at 3¾ hours after the birth of the first piglet, 3-4 hours actually consists of observations between 3 and 3¾ hours (prior to injection), and 4-5 consists of observations between 3¾ and 5 hours following the BFP (immediately post-injection).

3.3.5. Parameters Recorded

3.3.5.1. Posture

The posture of the sows was recorded during the observation periods (Table 1a).

3.3.5.2. Sow-piglet interactions

When a piglet approached the sows nose (within one piglet body length) the response of the sow was recorded. Whether the sow crushed or stood on a piglet, and if she responded to the tactile or vocal stimulation from that piglet was recorded (Table 1b).

Table 1. a) Description of the postures recorded and b) the categories of sow-piglet interactions recorded when a piglet was within one piglet body length of the sow's nose.

a)

Posture	**Description**
Stand	Upright with all four feet on the ground
Sit	Rear end on the floor with two front feet on the ground
Kneel	Front legs bent with two back feet on the ground
Lie Ventrally	Lying on udder with neither shoulder touching the ground
Lie Laterally	Lying with udder exposed and one shoulder completely on the ground

b)

Category	**Description of sow's response**
No response	no visible response to the piglet
Nose	actively touches the piglet with her nose
Bite	bites, or attempts to bite the piglet
Mouth	places her mouth over the piglet but does not bite
Root	pushes the piglet with her nose
Response to crush	crushes piglet and responds by getting up
No response to crush	crushes piglets and does not respond

3.3.5.3. Piglet activity and location

The time of birth, sex and weight of the piglets was recorded. Throughout each of the observation periods, when piglets were present, the location of the piglets was recorded at 5 minute intervals. The total number of piglets active (i.e. not lying motionless) was also recorded.

3.3.6. Statistical Analysis

3.3.6.1. Gestation Length and the Progress of Parturition

One way analysis of variance (Minitab, version 7.2) was used to compare gestation length, length of parturition, piglet interval, litter size and mean piglet weight between the two treatment groups.

3.3.6.2. Posture

The duration of each posture, and the number of times the sows changed posture was determined for each hour during farrowing. Posture duration and change was calculated for each of the pre- and post-farrowing days using a mean of the two hourly observation periods carried out on these days. Due to insufficient frequency and duration of sitting and kneeling, the effects of time and treatment on these postures could not be determined. The duration of each posture and the total number of posture changes were analysed using a repeated measures of analysis of variance (Genstat, Version 5, Lawes Agricultural Trust, Rothamsted Experimental Station) which was carried out on all observation periods to determine the effect of time. To ensure that there was no difference between the allocated groups prior to receiving the injection, an analysis of variance (Genstat, Version 5, Lawes Agricultural Trust, Rothamsted Experimental Station) was carried out on the days pre-farrowing and the first four hours following the birth of the first piglet (i.e. prior to the injection). Another repeated measures analysis of variance (Genstat, Version 5, Lawes Agricultural Trust, Rothamsted Experimental Station) was used to determine

treatment differences in the period following the injection. In all the above analyses length of parturition was used as a covariate and the significance level of the variance ratio was determined using a randomisation test (Genstat, Version 5, Lawes Agricultural Trust, Rothamsted Experimental Station). To determine the effects of naloxone, without confounding effects of length of parturition, post-hoc analysis included using a two sample t-test (Minitab, version 7.2) and a paired t-test (Minitab, version 7.2) on data from short parturition sows only. To determine effects of length of parturition, saline sows with short and long parturition duration were compared using paired and two sample t-tests (Minitab, version 7.2), thereby not involving animals receiving naloxone.

3.3.6.3. Sow-piglet interactions

A repeated measures analysis of variance (Genstat, Version 5, Lawes Agricultural Trust, Rothamsted Experimental Station) was used to determine effects of time and treatment on the frequency of sow-piglet interactions. Similarly to the posture data, the significance level of each variance ratio was determined using a randomisation test (Genstat, Version 5, Lawes Agricultural Trust, Rothamsted Experimental Station) To account for changes in the sows interactions with piglets in relation to the end of farrowing, length of parturition was used as a covariate. Similar to the posture data post-hoc analysis involved two sample t-tests and paired t-tests (Minitab, version 7.2) to determine effects of naloxone (short parturition sows only) and length of parturition (saline sows only).

3.3.6.4. Maternal Responsiveness

The most frequent interactions between the sows and their piglets were 'nose' (total 'nose' = 1764 out of 3364 total interactions) and 'no response' (total 'no response' = 1393 out of 3364 total interactions. Given the predominance of 'nose' and 'no response' the following index of maternal responsiveness was derived:

$$\text{Maternal Responsiveness} = \frac{\text{Nose} - \text{No response}}{\text{Nose} + \text{No response}}$$

(therefore, +1 = all noses; -1 = all no responses; 0 = equal number of nose and no response)

A repeated measures analysis of variance (Genstat, Version 5, Lawes Agricultural Trust, Rothamsted Experimental Station) was carried out over all the observation periods, and then before and after the injection. Similarly to the analysis of the individual interactions, length of parturition was used as a covariate. Again post-hoc analysis involved two sample t-tests and paired t-tests (Minitab, version 7.2) to determine effects of naloxone (short parturition sows only) and length of parturition (saline sows only).

3.3.6.5. Piglet Location and Activity

The number of piglets active as a proportion of the total number of piglets was analysed using a repeated measures analysis of variance (Genstat, Version 5, Lawes Agricultural Trust, Rothamsted Experimental Station) over the farrowing period and the days post-farrowing. The number of observations that the piglets were found at the sow's head, as a proportion of the total possible number of observations, was compared between the treatments during the six hours of farrowing, and then the days post-farrowing using a Mann-Whitney test (Minitab, version 7.2). In both these analyses length of parturition was accounted for.

3.4. Results

3.4.1. Gestation length and piglet information

There was no difference in length of gestation between the allocated groups (mean gestation length ± s.e. (days) = 116.1 ± 0.58 and 114.9 ± 0.70 for saline and naloxone animals respectively; $t_{13} = 1.38$, p=0.19). Piglet weight was not affected by treatment (mean piglet weight ± s.e. (kg) = 1.6 ± 0.11 and 1.6 ± 0.13 for saline and naloxone sows; $t_{13} = -0.26$, p=0.80).

3.4.2. Progress of Parturition

Those sows that received naloxone had shorter parturitions than those sows receiving saline (mean length of parturition ± s.e. (hours) = 5.41 ± 1.11 and 2.70 ± 0.34 for saline and naloxone sows, $t_8 = 2.36$, $p<0.05$). All sows receiving naloxone had finished farrowing prior to the injection and therefore the shorter parturition was not an effect of the administration of naloxone. Due to the length of parturition being unbalanced between treatments all subsequent data will be presented in terms of Short (< 4 hours) parturition sows, which includes 8 naloxone sows and 4 saline sows, and Long (> 4 hours) parturition sows which includes 4 saline sows. As there was no difference in the number of piglets born alive: 11.63 ± 1.32 and 10.63 ± 0.73 for saline and naloxone sows, the shorter parturition also resulted in a tendency for a shorter interval between piglets in the naloxone sows (mean piglet interval ± s.e. (minutes) = 31.0 ± 6.12 and 17.2 ± 2.69 for saline and naloxone sows; $t_9 = 2.07$, p = 0.068).

3.4.3. Posture Duration

3.4.3.1. Standing

The proportion of time spent standing decreased from the days pre-farrowing towards the third hour of farrowing (Figure 1a) in the short parturition sows. Levels in the long parturition sows remained elevated over the first three hours and decreased

towards the sixth hour of farrowing. The proportion of time spent standing then remained low over the days post-farrowing (overall time effect: $F_{10, 135} = 3.86$, $p<0.001$).

Regarding the short parturition sows only, the change in the proportion of time spent standing following the injection (Figure 1a) did not change significantly in the saline sows, however the rise from Hour 3-4 (pre-injection) to Hour 4-5 in standing in the naloxone sows was significant ($t_7=2.78$, $p<0.05$).

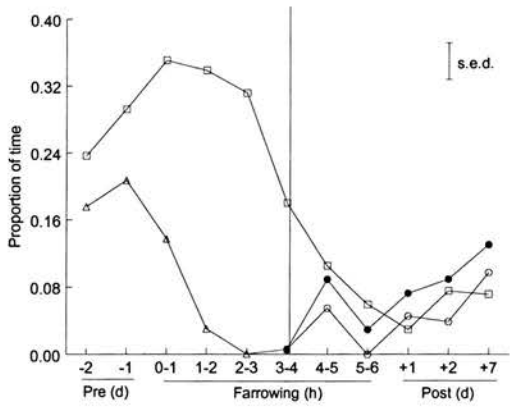
3.4.3.2. Lying Ventrally

A similar pattern was seen for the proportion of time spent lying ventrally in the short parturition and long parturition sows with levels decreasing from the days pre-farrowing and during farrowing itself and then increasing over the days post-farrowing (overall time effect: $F_{10, 135} = 8.65$, $p<0.001$) (Figure 1b). An interaction between time and treatment was found in the post-injection period ($F_{4,55}=2.94$, $p<0.05$) which was the result of the short parturition sows receiving naloxone spending a greater amount of time lying ventrally in hour 4-5 ($t_7=2.39$, $p<0.05$) and tending to in hour 5-6 ($t=_7 1.93$, $p=0.095$).

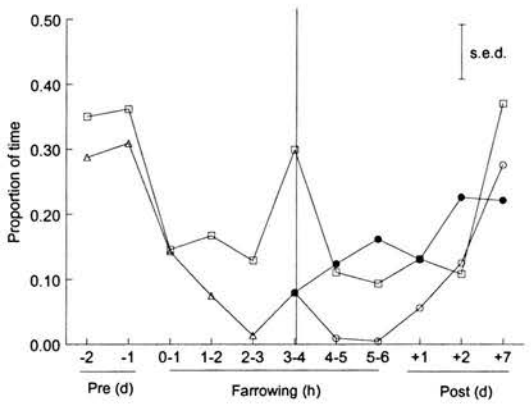
3.4.3.3. Lying laterally

Correspondingly lying laterally increased from the days pre-farrowing reaching a maximum for the short parturition sows during the third hour when these sows spent almost all of their time in this posture (Figure 1c). The long parturition sows showed an increase in lying laterally from the days pre-farrowing but spent considerably less time than the short parturition sows in this posture throughout the first six hours of farrowing (effect of length of parturition: $F_{10,135}=8.83$, $p<0.01$). Lying laterally remained high over the post-farrowing period (overall time effect: $F_{10, 136} = 10.21$, $p<0.001$). There was a tendency for short parturition sows receiving naloxone to spend less time lying laterally than short parturition sows receiving saline in the post-injection period ($F_{4,55}=2.23$, $p=0.078$).

a)



b)



c)

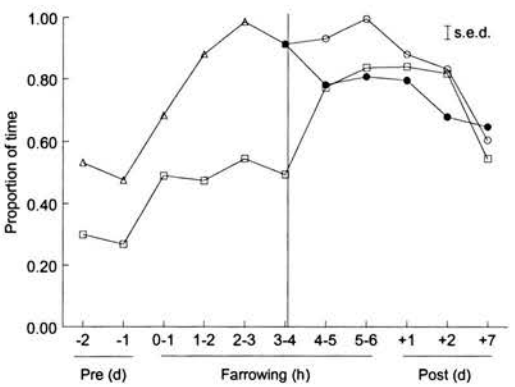


Figure 1. The proportion of time sows spent a) standing, b) lying ventrally and c) lying laterally over the days pre-farrowing (Pre-d), farrowing itself (hourly periods) and the days post-farrowing (Post-d). Open triangles (Δ) represent short parturition sows prior to injection (n=12) which was given at the end of hour 4 (indicated by vertical line), and then divided into two treatments; naloxone (●, n=8) and saline (○, n=4). Long parturition sows are shown separately and consists of saline treated sows only (□, n=4).

3.4.4. Posture Change

Overall posture change was affected by time ($F_{10, 134} = 4.47$, $p<0.001$). The number of posture changes increased in the first hour of farrowing in relation to the days pre-farrowing (Figure 2). Over hours 1-2, 2-3 and 3-4 the number of posture changes returned to pre-farrowing levels in short parturition sows (Figure 2). Similarly the long parturition sows showed an increase in posture changes in the first hour of farrowing however this continued to increase until hour 3 (effect of length of parturition over the pre-injection period: $F_{1,13}=16.14$, $p<0.001$). Once the injection had been given short parturition sows receiving naloxone and saline showed an increase in the number of posture changes however this was significant in the naloxone sows only ($t_7=3.02$, $p<0.05$). With regard to only the sows receiving saline, the number of posture changes during Hour 5-6 was greater for those sows with a longer parturition ($t_3 = 2.99$, $p<0.05$).

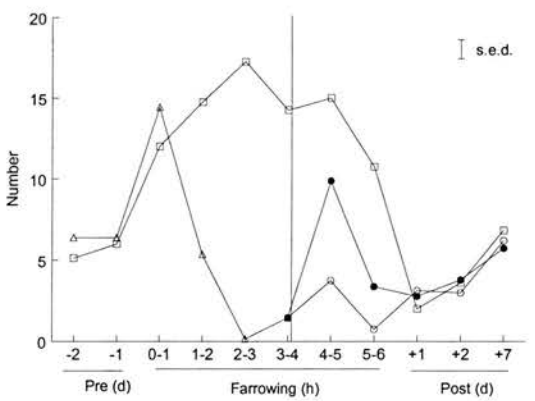


Figure 2. The number of posture changes made over days pre-farrowing (Pre-d), farrowing itself (hourly time periods) and the days post-farrowing (Post-d). Open triangles (Δ) represent all short parturition sows prior to the injection (n=12) which was given at the end of hour 4 (indicated by vertical line), and then divided into the two treatments; naloxone (●, n=8) and saline (○, n=4). Long parturition sows are shown separately and consists of saline treated sows only (□, n=4).

3.4.5. Sow-Piglet Interactions

The interactions with the greatest frequency were nose and no response and therefore formal analysis of the remaining interactions was not carried out due to the insufficiency of the data. Bite, although occurring relatively frequently (4%; (149/3364)), was not regarded as representative of the sample group as this response to piglets occurred mainly in four of the 16 sows and only during the first few hours of farrowing. Mouthing, Rooting, Response to crush and No response to crush occurred 8, 34, 1 and 15 times respectively during mainly the first few hours of farrowing and therefore were not regarded as representative of the sample or of the time periods observed.

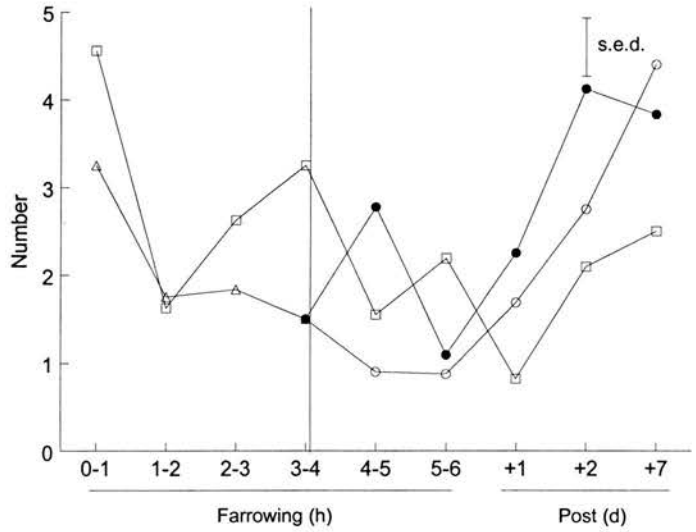
3.4.5.1. Nose

Nosing of piglets by the sow was affected by time ($F_{7,86}$=7.13, $p<0.001$) with the greatest number of nose contacts occurring in the first hour of farrowing, and on the second and seventh day following farrowing (Figure 3a). There was no difference in the number of nose contacts between the allocated groups prior to the injection, however a time x treatment interaction was found in the immediate post-injection period (hour 4-5 and 5-6; $F_{1,14}$=5.73, $p=0.05$) with naloxone treated short parturition sows performing more nosing of piglets than saline treated short parturition sows.

3.4.5.2. No response

A lack of response by the sow to piglets approaching her nose was also affected by time ($F_{11, 142}$ = 4.90, $p<0.001$) with the number of No responses increasing towards hour 3-4, and then decreasing over the remainder of farrowing and remaining at low levels over the days post-farrowing (Figure 3b). There was no difference in the number of no responses between the allocated groups prior to injection and naloxone had no effect on the number of No responses. There was also no effect of length of parturition on the frequency of No responses.

a)



b)

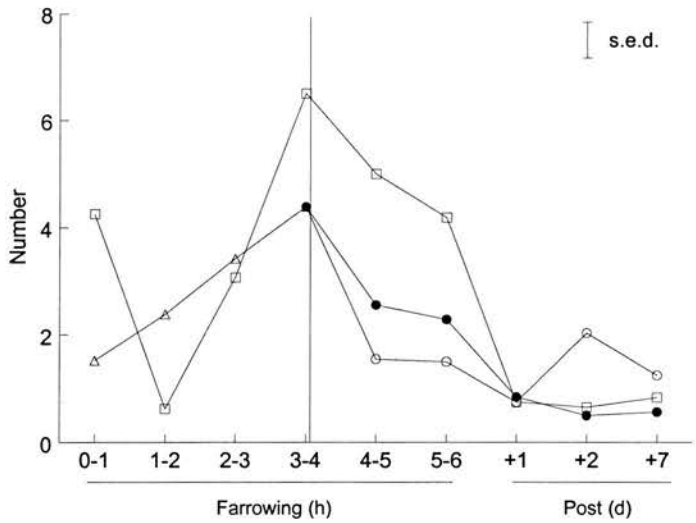


Figure 3. The number of a) Noses and b) No responses performed by the sow to her piglets over the farrowing period (hourly time periods) and the days post-farrowing (Post-d). Open triangles (Δ) represent all short parturition sows prior to the injection (n=12) which was given at the end of hour 4 (indicated by vertical line), and then divided into the two treatments; naloxone (●, n=8) and saline (○, n=4). Long parturition sows are shown separately and consists of saline treated sows only (□, n=4).

3.4.5.3. Maternal Responsiveness Index

The maternal responsiveness index (Figure 4) was positive during the first hour of farrowing, but became negative in the second hour and remained so until the end of hour 3-4, which preceded the injection (overall time effect: $F_{8,111} = 7.21$, $p<0.001$) (Figure 4). There was no difference between the allocated groups during this time in terms of injection or length of parturition. During the post-injection period there was an effect of the length of parturition with short parturition pigs being more responsive to their piglets ($F_{1,55}=5.64$, $p<0.05$). However post-hoc analysis showed that this was due to the confounding effects of naloxone as there was no difference in responsiveness between the long and short parturition saline sows (Hour 4-5: $t_5=-0.12$, $p=0.91$, Hour 5-6: $t_5=-0.23$, $p=0.83$).

Regarding the short parturition sows, during hour 4-5, the sows receiving saline continued to have a negative maternal responsiveness index, whereas the index of the naloxone treated sows became positive ($t_7=1.94$, $p=0.094$) (Figure 4) with this continuing during hour 5-6.

During the days post-farrowing the index for both treatments became positive, however there was a tendency for the naloxone animals to have a higher maternal responsiveness index (mean maternal responsiveness index = 0.23 and 0.07 for naloxone and saline sows respectively (s.e.d. = 0.09), $F_{2,27} = 2.55$, $p=0.097$). Post-hoc analysis showed this to be mainly due to naloxone treated sows having a higher maternal responsiveness index on day +2 ($t_4 = 2.30$, $p=0.083$) (Figure 4).

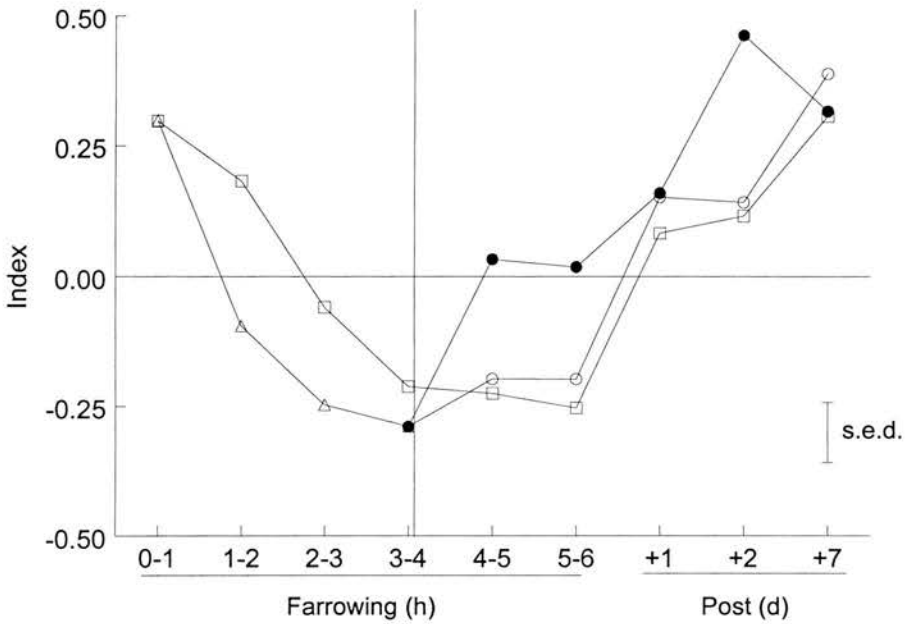


Figure 4. The maternal responsiveness index of the sows over the farrowing period (hourly time periods) and the days post-farrowing (Post-d). Open triangles (Δ) represent all short parturition sows prior to the injection (n=12) which was given at the end of hour 4 (indicated by vertical line), and then divided into the two treatments; naloxone (●, n=8) and saline (○, n=4). Long parturition sows are shown separately and consists of saline treated sows only (□, n=4). +1=all noses; -1=all no responses; 0=equal number of noses and no responses.

3.4.5.4. Piglet activity and location

There was no difference in the activity levels of the piglets of the naloxone and saline sows ($F_{1,13}$=0.86, p=0.371). There was also no difference in the proportion of piglets at the sows head during the farrowing period between the treatments ($F_{1,13}$=0.12, p=0.733). However it was found that piglets of sows receiving naloxone spent a greater proportion of observations at the sows head in the post-farrowing days (W=89.5, p<0.05).

3.5. Discussion

The results of this study confirm previous qualitative descriptions of posture during farrowing (Jones, 1966, Petersen et al., 1990, Randall, 1972) in that the sows in this study, which were housed in straw-bedded pens, after an initial period of investigating their piglets spent the greatest proportion of the farrowing period lying laterally. This reduction in activity coincided with increased passivity, characterised by sows reducing the number of times they responded when a piglet was present at their face. The changes in the maternal responsiveness index over the farrowing period reflects the reduction in behavioural responses to piglets from the initial stage of delivery. The reduction in sow activity appears to be associated with the cumulative effect of piglet deliveries, as the reduction in activity is somewhat delayed in longer parturition sows.

In the present study, naloxone significantly increased standing, lying ventrally and posture changes suggesting that opioids may be involved in the reduction in activity during farrowing. In addition the administration of naloxone caused an increase in nosing of piglets suggesting opioid involvement in the responsiveness of the sows to their piglets.

This opioid involvement in reduced activity and responsiveness may be explained by various potential routes of action. Opioids are known to be involved in an endogenous analgesic system at parturition in the rat (Gintzler, 1980) and the pig (Jarvis et al., 1997a) and it has been recognised that parturition in the pig appears to occur with relative ease (Jones, 1966b, Randall, 1972). Therefore opioids, through their analgesic properties, may reduce the perception of pain and thereby expressions of discomfort such as changes in posture. This reduction in pain perception may also prevent the association between the birth of piglets and pain, thereby reducing piglet-directed aggression.

Alternatively opioids may have their behavioural effects through a more general sedative effect. Exogenous opiates, such as morphine, are commonly used to induce sedation in veterinary practice (Jochle et al., 1991). Dogs treated with morphine show general social unresponsiveness whereas those treated with naloxone show increased social solicitation (Panksepp et al., 1983).

Finally, opioids have been shown to cause inhibition of oxytocin in response to acute stress during parturition in the rat (Leng et al., 1988) and the pig (Lawrence et al., 1992). As oxytocin is involved in maternal behaviour (Fahrbach et al., 1985, Pedersen and Prange, 1979, Pedersen et al., 1982), the effect of naloxone observed in this study may indicate an opioid inhibition of oxytocin during delivery in the pig which subsequently reduces maternal responsiveness.

The increased standing and posture changes seen in long parturition sows may be due to the prolonged piglet birth intervals as opioid release resulting from pain associated with the birth of a piglet may be reduced. The role of opioids in sows with longer parturitions can not be determined in this study due to all the long parturition sows being allocated to the saline group.

Under wild and free-ranging conditions the pre-parturient sow isolates herself from the group and builds a nest in which to give birth. She continues to nurse her piglets in the nest for around one week after birth (Jensen, 1986), perhaps reducing the need for immediate bonding with her piglets. This study suggests that opioids reduce mother-young bonding firstly, as the sows were generally unresponsive to their piglets during farrowing, and secondly that piglets of sows receiving naloxone were found in increasing numbers around the sow's head in the days following farrowing. Reduction in activity may be a greater priority in the initial stages of farrowing as posture changing by the sow is potentially hazardous for the piglets. In addition if the sow remains in lateral recumbency the udder is fully exposed and is providing a source of

nutrition and warmth for the piglets and is therefore a component of good maternal care (Fraser, 1984). Increased passivity may also be advantageous in an isolated nesting animal (Jensen, 1986) as remaining inconspicuous would prevent detection by predators. Reduced responsiveness may also be characterised by reduced vocalisations by the sow thus reducing piglet vocalisations. There may also be some opioid involvement in the vocalisations of the sow and her piglets as there is evidence to suggest an endorphin-mediated inhibition of distress vocalisations in adult guinea pigs (Herman and Panksepp, 1981). Therefore opioids may be part of the neural mechanism that increases the survival of piglets by trading-off increased passivity against early mother-young bonding.

In conclusion it appears that what is regarded as good maternal care in other animals may not represent adaptive maternal care in the sow. In order to maximise the number of piglets surviving the initial period after birth, passivity, which is characterised by lateral lying and unresponsiveness to piglets, appears to be an important aspect of maternal care in the sow, and this study indicates endogenous opioids as a neural substrate. There appears to be a delay in the reduction of activity and responsiveness in sows which have a prolonged parturition which may be due to attenuation of opioid levels resulting from a longer interval between the birth of piglets.

Chapter 4

The effect of environment on behavioural activity, ACTH, β-endorphin and cortisol in pre-farrowing gilts

4.1. Abstract

This study examined the temporal relationships between behavioural activity and hormones associated with stress in gilts farrowing in two environments. Thirty one Large White X Landrace gilts with indwelling jugular catheters were blood sampled daily (0800 and 1600) from 10 days before their expected parturition date (EPD). Five days before EPD they were moved to either a farrowing crate (C) with no bedding, or a pen (P) (2.5m x 3.0m) with straw provided and were blood sampled daily at 0800, 1200 and 1600. Around 12 hours before the onset of farrowing an extension was fitted to the catheter and blood samples were taken remotely at ½ hour intervals. The posture of the gilts was recorded using five minute scan samples over the 24 hours pre-farrowing. The proportion of scans standing (an index of activity) was strongly affected by time ($p<0.001$) with peak levels at approximately eight hours pre-farrowing in both treatments, and by treatment (0.25 v. 0.33 for C and P gilts respectively; s.e.d. 0.03, $p<0.05$). Plasma cortisol concentrations also rose before farrowing ($p<0.001$) reaching a peak at 12-6 hours pre-farrowing. Crated gilts had higher cortisol concentrations than penned gilts (overall mean: 41.5 v. 30.7 ng/ml for C and P gilts respectively; s.e.d. 3.8, $p<0.05$) at 24-12 ($p<0.05$), 12-6 ($p<0.01$) and 6-2 ($p<0.05$) hours pre-farrowing. Plasma ACTH concentration showed a similar pattern to cortisol over the pre-parturient period, peaking at 12 hours pre-farrowing in both treatments (time: $p<0.001$); crated gilts had significantly higher concentrations of ACTH at six hours pre-farrowing only ($p<0.05$). Plasma β-endorphin concentrations also showed a gradual rise ($p<0.001$) towards parturition; however no treatment differences were seen. These results suggest that the pituitary-adrenal (PA) axis is stimulated during pre-farrowing activity irrespective of farrowing environment. Crates, without bedding, further stimulate the PA axis over the pre-farrowing period perhaps by preventing nest-building. The rise in ß-endorphin may be involved in an endogenous defence against parturition pain.

4.2. Introduction

When pre-parturient pigs are given the opportunity, they will isolate themselves from the herd and select a site in which to build a nest. Nest-building in free-ranging pigs consists of digging a hole and placing grass and other soft materials into it; a process which can take several hours (Jensen, 1986). In commercial production sows commonly farrow in a crate environment. Pigs in these conventional farrowing crates continue to show behaviours indicative of nest-building during the pre-parturient period; an increase in activity is seen (Meunier-Salaun *et al.*, 1991), and also in floor and fixture-directed behaviour (Lawrence *et al.*, 1994). Nest-building behaviours appear to be performed by pigs in a barren environment and by naive gilts suggesting that there is an internal stimulus. This is further reinforced by the continuation of nest-building behaviour in sows when presented with a pre-formed nest (Arey *et al.*, 1991).

A study by Castren *et al.*, (1993) suggested a possible hormonal control in that the initiation of nest-building occurred due to the pre-parturient rise seen in prolactin. However a study by Lawrence *et al.*, (1994) did not find a convincing relationship between prolactin and substrate-directed behaviour, with some gilts performing nest-building behaviour and showing no rise in prolactin.

In addition to an internal stimulus for nest-building, there are also external factors which can modify the behaviours performed. The substrates available to the sow are of great importance as when offered a choice of farrowing sites sows will always choose a strawed environment as their nesting site (Arey *et al.*, 1989). Repeated provision of sawdust to young sows causes an increase in activity and behaviours characteristic of nest-building (Cronin *et al.*, 1993), suggesting that substrates in the environment modify nest-building. However within conventional farrowing systems substrates are often lacking. It has been suggested that pre-parturient sows are not highly motivated to obtain straw (Hutson, 1988), however the sows used in this

particular study were multiparous and had no prior experience of straw-bedding thus rendering it a novel substrate.

A number of studies have previously reported that cortisol rises in sows around parturition however these studies were all conducted using conventional farrowing crate systems (Killian *et al.*, 1973, Molokwu and Wagner, 1973). In a comparative study contrasting the response of gilts to crates and pens, gilts housed in crates with no bedding had higher concentrations of plasma cortisol relative to gilts housed in straw-bedded pens over the pre-parturient period (Lawrence *et al.*, 1994). Within this study the crated gilts were also found to spend less time standing and more time lying laterally, and they also performed more fixture-directed behaviour than penned gilts, perhaps due to lack of straw. One interpretation of these results is that increased concentrations of maternal cortisol in the pre-farrowing period reflect interference of nest-building by the crate rather than the parturition process itself.

Plasma cortisol is commonly used as an indicator of physiological stress, and the aim of this study was to determine the relationship between plasma cortisol and activity of pre-parturient gilts in two environments. In addition, to fully test the hypothesis that crates without bedding specifically stimulate the pituitary-adrenal (PA), we extended our measurements to include plasma ACTH and β-endorphin which are released concomitantly from the pituitary (Guillemin *et al.*, 1977).

Therefore the overall aim of this study was to examine the effects of farrowing environment on stimulation of the (PA) axis, and to investigate any relationship with changes in pre-parturient activity.

4.3. Animals, Materials and Methods

4.3.1. Animals

The subjects of this study were 32 Large White x Landrace primiparous females (gilts; Cotswold Pig Development Co. Lincoln, UK). The gilts were purchased, at approximately six months of age, in eight consecutive groups (n=4) and each group was housed in a straw-bedded pen (2.6 m x 4.1 m). The gilts were fed 2.5 kg/day of a commercial diet providing 13 MJ DE/kg, and the pens were cleaned as required with straw being provided for bedding twice a week. After a four week period a boar was introduced to the pen daily and the gilts were served on two consecutive days. The expected parturition day (EPD) was calculated as 114 days after the first service date. Once pregnancy was confirmed at around 32 days after service the gilts were housed in groups in a strawed yard (9.6m x 6m) where they were floor fed 2.5 kg/day of the same commercial diet. Similarly the pens were cleaned and straw for bedding provided twice a week.

4.3.2. Catheterization

All gilts had a jugular catheter (silastic, Osteotec Ltd., Christchurch, Dorset, U.K., internal diameter - 1.47 mm and external diameter - 1.93 mm) implanted under general anaesthesia around 15 days before the EPD (15 ± 0.66 days) (for full details of the procedure see Lawrence et al, 1992). Briefly, the catheter was protected with an adhesive neck bandage, and a connecting tap at the back of the neck was used for the removal of blood. The catheters were flushed daily with saline and primed with heparinised saline (150 I.U./ml) until sampling began 10 days before EPD. After the operation, the gilts were housed individually in straw-bedded pens (2 m x 2 m). The same commercial feed was offered in two meals at 0800 and 1600 h (2.5 kg/day). One gilt was removed from the study due to a blocked catheter.

4.3.3. Experimental Housing

Five days before EPD the gilts were weighed and moved to either a conventional farrowing crate (Treatment C (n=15) - 2.25 m in length, 0.45 m in width and 1.05 m in height) or to a pen (Treatment P (n=16) - 2.5m x 3.0m). The crate consisted of a solid floor with slatted dunging area at the back and no straw was provided. The pen had a solid floor which was sloped to allow drainage and straw was provided. Both environments had a creep area outwith the specified dimensions. The temperature of the housing was controlled (mean minimum temperature (°C) ± s.e.m. = 15.16 ± 0.05; mean maximum temperature (°C) ± s.e.m. = 18.24 ± 0.05), and the lights were on between 0800 and 1600. Dim lights were used to allow observation during the night. The gilts were offered 3 kg/day of a food that provided 13.75 MJ DE/kg and contained 18% protein in 2 meals at 0800 and 1600. After parturition the food level was gradually increased to appetite. The pens were cleaned and fresh straw provided after the morning feed, and the slatted area in the crates was also cleaned at this time.

4.3.4. Blood Sampling

The blood sampling procedure (Table 1) began 10 days before EPD (day -10) and continued until five minutes after the whole body of the first piglet (P1) had been expelled by the gilt. One sample was also taken two days after farrowing. Samples were collected directly from the tap at the neck until the onset of nest-building behaviour (See Section 2.6) when a silastic extension tube was fitted. The catheter extension minimised the disturbance to the gilts by allowing samples to be taken from outside the crate or pen area. Saline was used to replace the volume of blood taken and the catheter and extension primed with heparinised saline (75 I.U./ml). Heparinised monovette tubes (Sarstedt, Leicester, UK.) were used to collect blood samples. The samples were kept at 4°C for 30 minutes before centrifuging. They were then spun at 3000 r.p.m. at 4°C for 20 minutes. Aliquots of plasma were pipetted and stored at -20°C for later assay.

Table 1 - Description of samples used in time periods for (a) cortisol and β-endorphin and specific time points for (b) ACTH. pr-m = pre-move: baseline samples taken before movement into the allocated farrowing environments, po-m = post-move: samples taken on the day of the move (day -5) to the allocated farrowing environment, m+1 = samples taken during the day after the move to the allocated farrowing environment. P1 = birth of the first piglet. Numbers in time columns relate to hours pre-farrowing.

a) Cortisol and β-endorphin

Time	**Blood samples used in analysis**
pr-m	Day -10 1600, Day -6 1200, 1600
po-m	Day -5 1200, 1600
m+1	Day -4 0800, 1200, 1600
-48/-24	4 hourly samples (0800,1200,1600)
-24/-12	4 hourly samples (0800,1200,1600)
-12/-6	Every 30 minutes
-6/-2	Every 30 minutes
-2/-1	Every 10 minutes
-1/0	Every 10 minutes
0	P1 and P1+ 5 minutes

b) ACTH

Time	**Blood samples used in analysis**
pr-m	Day -6 1200
-48	-48 hours
-24	-24 hours
-12	-12 hours
-10	-10 hours
-8	-8 hours
-6	-6 hours
-4	-4 hours
-2.5	-2.5 hours
-1	-1 hour
+2 days	1200 on day +2

4.3.5. Hormonal Analysis

Blood samples from different groups and treatments were balanced across assay runs. Plasma used for all assays had been thawed once only.

4.3.5.1. Cortisol. Cortisol was extracted using diethyl ether from 100µl of plasma and concentrations were measured using radioimmunoassay of the extracted steroid (Duncan *et al.*, 1990). The intra and interassay coefficients of variation were 11.25% and 14.96% respectively, and the minimum detectable level of the assay was 0.23 ng/ml. Single samples were extracted and then assayed in duplicate.

4.3.5.2. β-endorphin. 200µl aliquots of plasma were assayed in duplicate using radioimmunoassay. Dextran-charcoal was used in the separation process and the supernatant counted using a multigamma counter (Ebling and Lincoln, 1987). Intra and interassay coefficients of variation were 15.04% and 12.73% respectively, and the minimum detectable level of the assay was 49 pg/ml.

4.3.5.3. ACTH. ACTH concentrations were measured in single aliquots of 100µl of plasma using an immunoradiometric assay kit (Euro Path Ltd., Bude, Cornwall, UK). Second antibody method was used, the samples spun and the pellet counted using a multigamma counter (Brooks, 1992). The minimum detectable level of the assay was 5.0 pg/ml and the intra-assay coefficient of variation was 8.32%.

4.3.6. Behavioural Observations

During the 48 hours before the EPD the behaviour of individual gilts was recorded on 24 hour time-lapse video to enable general activity to be recorded. The posture of the gilts was recorded at five minute intervals during the 24 hours pre-farrowing. Four categories of posture were recorded: stand, sit, lie ventrally and lie laterally.

4.3.7. Statistical Analysis

4.3.7.1. Hormonal Measures. The results of the assays were divided into time periods (Table 1a and 1b). The data were normalised by log transformation and analysed using a repeated measures analysis of variance (ANOVA; Genstat version 5), with a blocked structure for pig and time period. Factors were treatment (two levels - crate and pen), group (eight levels) and time period (10 levels for cortisol and β-endorphin (Table 1a), and 11 levels for ACTH (Table 1b)). Post-hoc analysis was carried out where a significant time or treatment effect, or a time x treatment interaction was found. Comparison between treatments was made by t-tests (Minitab version 7.2) of mean values for each pig at individual time periods. When looking at time effects within treatments, paired t-tests (Minitab version 7.2) were used to test whether the mean difference between each time period and the baseline (pre-move) was significantly different from zero.

4.3.7.2. Behavioural Observations. Data obtained from the 5 minute scan samples were pooled into half-hour time periods for analysis. The proportion of observations within each half-hour was calculated for standing, sitting, lying ventrally and lying laterally. A repeated measures analysis of variance (ANOVA, Genstat 5) was used to analyse these data to determine treatment and time effects. Factors were treatment (two levels - crate and pen), group (eight levels) and time (48 levels).

4.3.7.3. Relationship between activity and hormonal levels. To determine whether there was any relationship between activity and hormonal levels a correlation of activity and cortisol, and activity and β-endorphin was carried out for each individual gilt using data from all time periods. A t-test (Minitab, version 7.2) was then used to determine whether the correlation coefficients for all the gilts were significantly different to zero, and then a further t-test was carried out to compare the correlation coefficients between the two environmental treatments.

4.4. Results

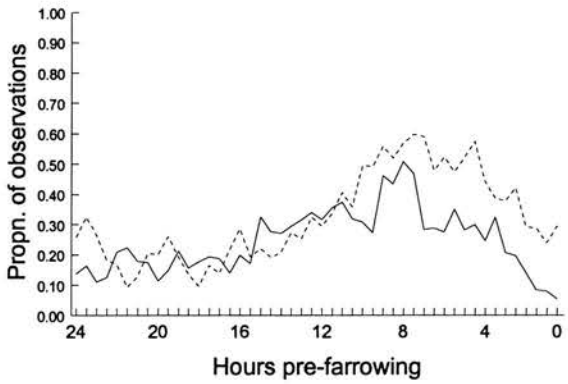
4.4.1. Gestation duration and weight of gilts

The average gestation length of the gilts was not affected by the farrowing environment (mean gestation length (days ± s.e.m.) = 113.47 ± 0.30 and 113.44 ± 0.33 for crate and pen animals respectively). The average weight of the gilts on entrance to their allocated farrowing environment was 200.2 kg ± 3.8 (s.e.m.) for crate animals and 200.6 kg ± 3.0 (s.e.m.) for the animals in pens.

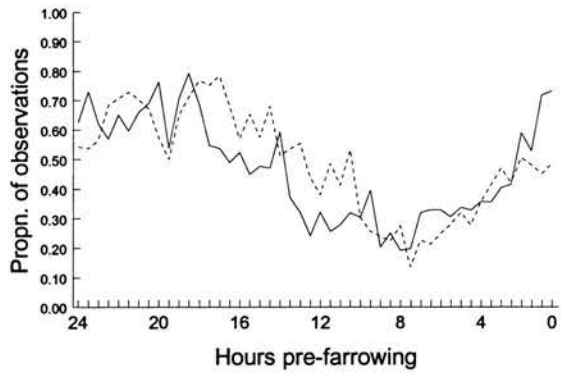
4.4.2. Behaviour

The posture of the gilts over the 24 hours pre-farrowing showed changes as parturition approached. Standing was strongly affected by time ($F_{47,573} = 6.73$, $p<0.001$) and increased from approximately 16 hours pre-farrowing in both treatments reaching a peak at eight hours pre-farrowing (Figure 1a). Crated gilts were observed standing for fewer of the observations (0.25 v. 0.33 for crate and pen gilts respectively; s.e.d 0.03) resulting in a treatment effect ($F_{1,15} = 5.52$, $p<0.05$). In contrast to standing, lying laterally decreased at around 16 hours pre-farrowing and reached a low at approximately eight hours pre-farrowing (Figure 1b) (time effect: $F_{47,573} = 10.53$, $p<0.001$). Lying ventrally and sitting changed with time, and sitting was also affected by treatment ($F_{1,15} = 52.56$, $p<0.001$) with crated animals sitting for a greater proportion of observations (0.10 v. 0.03 for crate and pen gilts respectively; s.e.d. 0.01); (Figure 1c).

a)



b)



c)

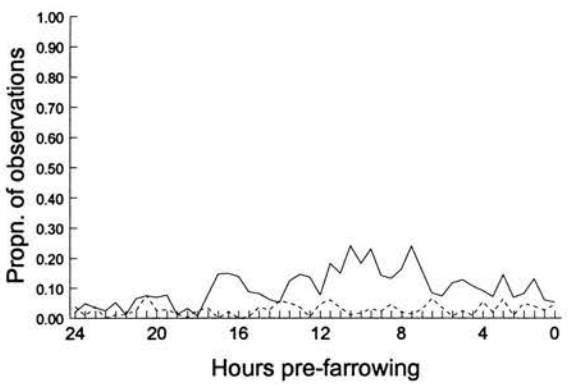


Figure 1 - Changes in the proportion of observations spent a) standing, b) lying laterally and c) sitting in crated gilts (__, n=15) and penned gilts (---, n=16) over the pre-farrowing period.

4.4.3. Cortisol

Before being moved to the farrowing environment circulating plasma cortisol concentrations did not differ between gilts allocated to the two environments, but were elevated in both treatments following the move (see Figure 2), with the elevation of cortisol being greater in crated gilts (treatment: $t_{(25)} = 2.09$, $p<0.05$). The increase seen in both crated and penned gilts was significant within treatment compared to pre-move values (see Table 2a). Cortisol concentrations fell in both treatments within one day of the move to concentrations which were not significantly different either between treatments or to pre-move levels.

A similar general trend in changes of plasma cortisol concentration was seen in both crated and penned gilts as parturition approached (time: $F_{9,119} = 18.06$, $p<0.001$), however crated gilts showed higher levels of plasma cortisol than penned gilts during much of the pre-parturient period (see Figure 2); (treatment: $F_{1,15} = 7.23$, $p<0.05$).

The crated gilts showed a non-significant rise in plasma cortisol concentrations at -24/-12 hours (Table 2a) whilst levels in the penned animals remained steady, resulting in a significant difference between treatments at this time ($t_{(16)} = 2.28$, $p<0.05$). Both treatments showed an increase in plasma cortisol concentration at -12/-6 hours (Table 2a), but the rise was greater in crated gilts ($t_{(21)} = 3.10$, $p<0.01$). Plasma cortisol concentrations in crated gilts started to decrease at -6/-2 hours, however continued to remain significantly higher in comparison to penned gilts ($t_{(22)} = 2.61$, $p<0.05$).

Each treatment continued to have significantly higher than baseline concentrations of plasma cortisol until the birth of the first piglet (see Table 2a), however there were no treatment differences over this time period.

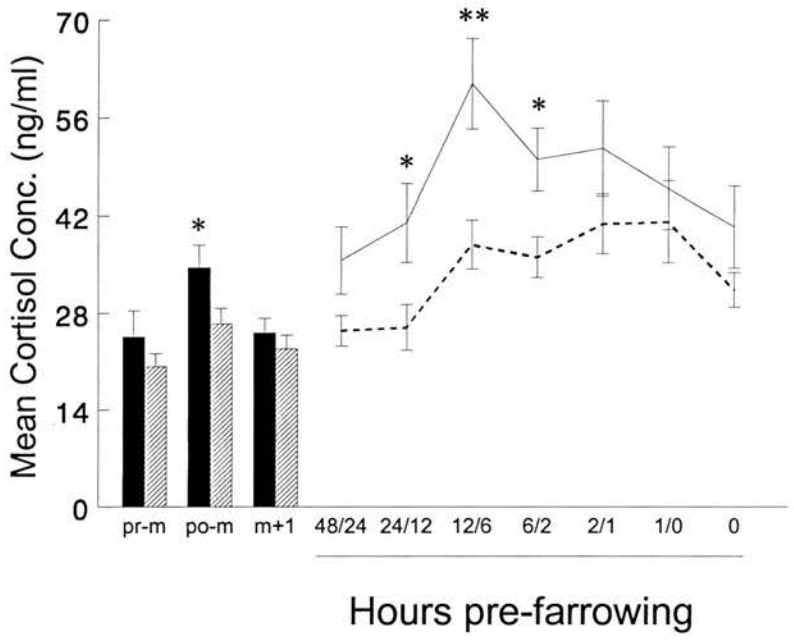


Figure 2 - Mean plasma cortisol concentrations of crated (■, __, n=15) and penned (▨, ---, n=16) gilts over the pre-farrowing period. pr-m = pre-move: baseline samples taken before movement into the allocated farrowing environments, po-m = post-move: samples taken at 1200 and 1600 on the day of the move (day -5) to the allocated farrowing environment, m+1 = samples taken during the day after the move to the allocated farrowing environment. Significance levels refer to differences between treatments at specific time periods.

Table 2 - Changes in plasma (a) cortisol, β-endorphin and (b) ACTH concentration over the pre-farrowing period in relation to a baseline (**pre-move** = 100%) for the two environments. pr-m = pre-move: baseline samples taken before movement into the allocated farrowing environments, po-m = post-move: samples taken on the day of the move (day -5) to the allocated farrowing environment, m+1 = samples taken during the day after the move to the allocated farrowing environment. Numbers in time columns relate to hours pre-farrowing.

a)

	Cortisol (%)		**β-endorphin (%)**	
Time	**Crate**	**Pen**	**Crate**	**Pen**
pr-m	**100**	**100**	**100**	**100**
po-m	140 *	131 *	108	95
m+1	105	110	125	108
-48/-24	135	124	87	121
-24/-12	157	129	97	103
-12/-6	247 ***	188 ***	147 *	127
-6/-2	206 ***	176 ***	133 **	144 *
-2/-1	212 **	202 **	129 *	138 *
-1/0	189 **	204 **	136 *	140 *
0	164 *	155 **	165 *	177 **

b)

	ACTH (%)	
Time	**Crate**	**Pen**
pr-m	**100**	**100**
-48	134 *	130 *
-24	169 *	163
-12	366 ***	256 **
-10	341 ***	219 *
-8	321 ***	238 **
-6	325 ***	190 *
-4	310 ***	197 *
-2.5	248 ***	184 *
- 1	268 **	208 **
+2 days	136 *	130 *

4.4.4. ACTH

Plasma ACTH concentrations did not differ between treatments before the gilts were moved into their allocated farrowing environment (see Figure 3).

A similar general pattern of plasma ACTH can be seen in both treatments, with an increase from -48 hours until reaching a peak at -12 hours. From this peak at -12 hours ACTH levels declined gradually until the onset of parturition (see Figure 3); (overall time effect $F_{10,213} = 20.83$, $p<0.001$). Over the pre-farrowing period the crated gilts showed higher concentrations of plasma ACTH however this difference was not significant at the 5% level. There was however a treatment effect on the pattern of plasma ACTH over time ($F_{10,213} = 1.94$, $p<0.05$) with crated gilts having higher concentrations at -6 hours ($t_{(22)} = 2.23$, $p<0.05$).

A significant increase in plasma ACTH occurred by 48 hours in both treatments compared to the baseline (pre-move). From -12 hours until the birth of the first piglet, plasma ACTH concentrations were significantly higher than baseline within each environment however this was at a higher significance level in crated gilts (see Table 2b).

A sample taken two days after parturition show a fall of plasma ACTH concentration in both treatments to a level comparable with those seen two days pre-farrowing.

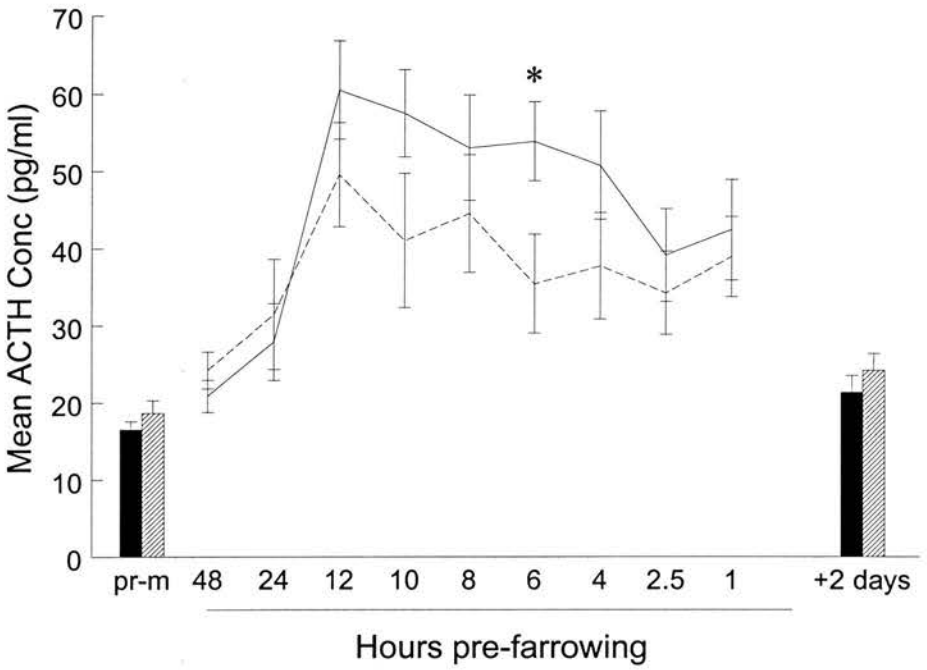


Figure 3 - Mean plasma ACTH concentrations of crated (■, __, n=15) and penned (▨, ---, n=16) gilts over the pre-farrowing period. pr-m = pre-move: baseline samples taken before movement into the allocated farrowing environments. +2 days = a sample taken two days after farrowing. Significance levels refer to differences between treatments at specific times.

4.4.5. β-endorphin

Plasma β-endorphin concentrations gradually increased over the pre-parturient period in both treatments (time: $F_{9,95} = 12.19$, $p<0.001$). A peak in plasma β-endorphin levels was seen in crated gilts at -12/-6 hours, however this was not significantly different to penned gilts at this time. A time x treatment interaction was found ($F_{9,95} = 2.09$, $p<0.05$), however post-hoc analysis showed that plasma β-endorphin concentrations were not significantly different between treatments at any specific time period (see Figure 4). Both treatments showed a rise in β-endorphin levels at the birth of the first piglet.

By using pre-move levels as a baseline, significant increases were seen in crated gilts (Table 2a), at -12/-6 hours which continued until the birth of the first piglet. The penned gilts did not show significant increases from the baseline levels until -6/-2 hours, and this also continued until parturition (Table 2a).

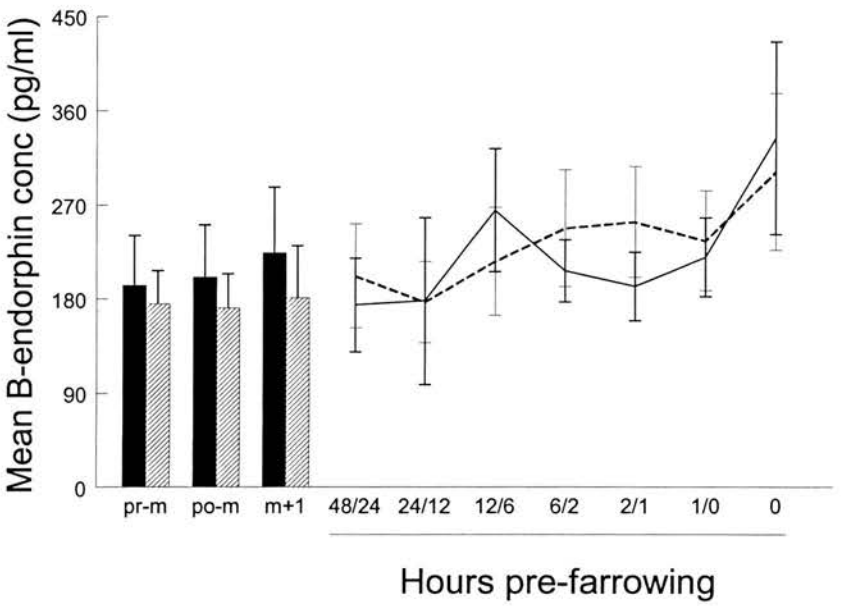


Figure 4 - Mean plasma β-endorphin concentrations of crated (■, __, n=15) and penned (▨, ---, n=16) gilts over the pre-farrowing period. pr-m = pre-move: baseline samples taken before movement into the allocated farrowing environments, po-m = post-move: samples taken at 1200 and 1600 on the day of the move (day -5) to the allocated farrowing environment, m+1 = samples taken during the day after the move to the allocated farrowing environment. Significance levels refer to differences between treatments at specific time periods.

4.4.6. Relationship between activity and hormonal levels

Activity and β-endorphin. No significant relationship was found between β-endorphin and activity either overall (mean r = 0.109, ns. (n=31)), or when comparing treatments (mean r = 0.113, ns. and 0.106, ns. for crate (n=15) and pen (n=16) gilts respectively).

Activity and Cortisol. Changes in plasma cortisol and activity were positively correlated over the 24 hours pre-farrowing (mean. r = 0.23, $p<0.01$ (n=31)), however when comparing treatments, only crated gilts showed a relationship between cortisol and activity (mean. r = 0.28, $p<0.05$ and 0.18, ns. for crate (n=15) and pen (n=16) gilts respectively).

4.5. Discussion

Similarly to other studies of pre-parturient behaviour in the pig (Meunier-Salaun *et al.*, 1991, Lawrence *et al.*, 1994), this study found that the time spent standing increased in all gilts irrespective of environment. The proportion of time spent standing began to rise at around 16 hours pre-parturition and reached a peak at eight hours pre-parturition, which was within the nest-building period defined by Castren *et al.*, (1993). It has been suggested that nest-building behaviour is under internal control (Castren *et al.*, 1993, Arey *et al.*, 1991) as pre-parturient sows, when presented with a pre-formed nest will continue to nest-build. Nest-building also appears to be modified by external stimuli as sows will choose a straw area, in preference to a hollow or a nest box, as their farrowing site (Arey *et al.*, 1989). This suggests that nesting material, as an external stimulus, is important to the pre-parturient sow, and it has been shown that the absence of nesting material in crated sows increases the proportion of time spent sitting (Cronin *et al.*, 1993). Similarly in the present study, the proportion of time spent sitting was greater in the crated gilts, with no bedding, compared with those in a straw-penned environment. This posture may be an expression of the gilt's motivation, but inability, to perform nest-building, or alternatively, may be a posture that crated gilts adopt more frequently.

Crated gilts showed a greater response to introduction to the farrowing environments as they had significantly higher concentrations of plasma cortisol than penned gilts at this time. This may be due to the increased novelty of the crate, however this was short-lived as the elevated concentrations of cortisol had returned to a non-significant level within 24 hours of the move. Over the 24 hours pre-farrowing, which is the period of greatest activity, the crated gilts had elevated concentrations of plasma cortisol and ACTH. This physiological stress may be due to the restrictive nature of the crate and the lack of substrate preventing the gilts from performing nest-building. The poor correlation between activity and plasma concentrations of cortisol observed

in this study may be due to the restriction of behaviour not resulting in immediate changes in hormonal levels. In the present study, both cortisol and ACTH increased irrespective of environment which, in terms of cortisol, is in contrast to a previous study by Lawrence *et al.*, (1994). Two possible suggestions for this rise in cortisol and ACTH are that the pre-farrowing period is stressful, or that the straw-bedded pen used in this study is not an optimal environment for a pre-parturient gilt. Work by Jensen (1986) has shown that free-ranging pigs isolate themselves from the group in the two days before farrowing and normally select a covered area in which to build their nest. Therefore the elevated concentrations of plasma cortisol and ACTH seen in all gilts in this study may be partly accounted for by lack of isolation or cover.

As ACTH causes the release of cortisol from the adrenal cortex, it may be expected that the difference between the two environments would be similar in terms of plasma ACTH and cortisol. However, in this study there was much less of a difference between the plasma ACTH levels of the crated and penned gilts than that of plasma cortisol. Work in rats has shown that there is a change in the relationship between cortisol and ACTH during pregnancy with the amount of cortisol present, at a given concentration of ACTH, increasing towards parturition (Atkinson and Waddell, 1995). However this does not account for the treatment differences seen in the present study. A possible explanation may be that ACTH has been shown to have increased corticosteroidogenic activity resulting in elevated levels of cortisol, in rats in a stressful situation (Goverde *et al.*, 1993). Therefore in this study, plasma ACTH may have been rendered more biologically active by the stressful crate environment thus resulting in elevated plasma cortisol.

In this study plasma β-endorphin gradually increased in all gilts over the pre-farrowing period, reaching a peak at the birth of the first piglet. Plasma β-endorphin is known to increase in humans during labour (Facchinetti *et al.*, 1983, Fettes *et al.*, 1984, Hoffman *et al.*, 1984, Kofinas *et al.*, 1985, Fajardo *et al.*, 1994, McLean *et al.*,

1994) as does ACTH (Fettes *et al.*, 1984, Fajardo *et al.*, 1994). The rise in plasma β-endorphin may be involved in producing analgesia at peripheral sites in preparation for the onset of parturition. Peripheral sites for opioid analgesia have been demonstrated in the rat using paw inflammation (Joris *et al.*, 1987). Studies involving epidural anaesthesia (Raisanen *et al.*, 1984) and physical conditioning (Varrassi *et al.*, 1989) in pregnant women suggest that there is an inverse relationship between plasma β-endorphin and pain perception.

In conclusion, this study has demonstrated that the pituitary-adrenal (PA) axis is stimulated during pre-farrowing activity irrespective of environment, which suggests that either the pre-farrowing period is stressful *per se*, or that neither environment used in this study is optimal for a pre-parturient gilt. Further stimulation of the PA axis occurs in pre-parturient gilts housed in farrowing crates suggesting that, through the restrictive nature of the environment and the lack of substrate, reduced ability to perform nest-building behaviours causes physiological stress. The increased plasma opioid, β-endorphin, observed in gilts in both environments may be involved in the alleviation of pain during parturition by acting at peripheral sites.

Chapter 5

The effect of environment on plasma cortisol and β-endorphin in the parturient pig and the involvement of endogenous opioids.

5.1. Abstract

Previous work has indicated that plasma cortisol increases during farrowing in the pig suggesting increasing physiological stress. The aim of this study was to determine changes in plasma cortisol and β-endorphin over farrowing in the pig to obtain a more detailed profile of pituitary and adrenal release at this time and also to investigate the involvement of endogenous opioids in the mediation of the HPA axis. Indwelling jugular catheters were implanted, under general anaesthesia, in 31 Large White X Landrace gilts approximately 15 days before the expected parturition day (EPD). Gilts were moved into either a farrowing crate, without straw (n=15), or a straw-bedded pen (n=16) 5 days before the EPD. Samples were taken during the pre-farrowing period and then during farrowing itself. At 7.5 minutes after the birth of the first piglet gilts either received naloxone, an opioid antagonist, (1 mg kg^{-1} body weight, i.v.) or a control dose of saline. Plasma β-endorphin increased following the birth of the first piglet but remained fairly constant over the third and fourth hour of farrowing. Plasma cortisol continued to increase over the four hours following the birth of the first piglet. Changes seen in these hormones were generally insensitive to the environment and there was little evidence of opioid mediation of the HPA axis at parturition. From these results it is suggested that certain aspect(s) of parturition itself stimulate the HPA axis. However it is unknown if the rise in plasma cortisol is a result of some stress-inducing factor of the parturition process or whether it reflects a metabolic function The study also demonstrates the lack of any inhibitory mediation of the HPA axis by endogenous opioids at parturition.

5.2. Introduction

Previous work examining plasma cortisol concentrations during the pre-farrowing period has shown that crates without straw cause increased physiological stress in comparison to a straw-bedded penned environment (Jarvis et al., 1997b). A reduction in the proportion of time spent standing in pre-parturient crated gilts (Jarvis et al., 1997b) suggests that this restrictive environment may cause physiological stress by prevention of the highly motivated nest-building behaviour normally seen at this time. However during farrowing itself it has previously been shown that plasma cortisol after the first hour of farrowing is not affected by the environment either during (Lawrence et al., 1994) or in the days post-farrowing (Cronin et al., 1991, Lawrence et al., 1994). A reduction in activity has been seen in sows once parturition has begun in both restrictive environments (Jones, 1966b, Randall, 1972, Jarvis et al.,unpublished data) and free-ranging environments (Jensen, 1986) with sows spending greater amounts of time lying laterally. Therefore the lack of the effect of environment on cortisol levels during farrowing and on the days post-farrowing may be due to the general reduction in activity and to the restriction imposed by the crate being effectively lessened. However although the activity of sows is reduced during farrowing, plasma cortisol continues to rise as parturition progresses (Lawrence et al., 1994) suggesting that some aspect(s) of parturition cause increasing physiological stress.

It is known that opioids inhibit oxytocin in the rat (Bicknell and Leng, 1982) and the pig (Lawrence et al., 1995) during parturition, and an opioid-mediated analgesic mechanism has been described in the pig at this time (Jarvis et al., 1997a). Previous work suggests that opioids may be involved in mediating the hypothalamic-pituitary-adrenal axis (Rushen et al., 1995) and therefore elevated opioid levels at parturition may be involved in mediating pituitary and adrenal release.

Therefore this study aims to determine the temporal profile of plasma cortisol and β-endorphin over the farrowing period as a measure of pituitary-adrenal activity in crated and penned gilts. In addition the study aims to determine the effects of endogenous opioids on the pituitary-adrenal axis by the administration of naloxone, an opioid antagonist.

5.3. Animals, Materials and Methods

5.3.1. Animals

Thirty two Large White x Landrace primiparous females (gilts; Cotswold Pig Development Co. Lincoln, UK) were the subjects of this study which lasted 12 months. The gilts were purchased, at approximately 6 months of age, in 8 consecutive groups (n=4) and each group housed in a straw-bedded pen (2.6 m x 4.1 m) under natural light and temperature. The gilts were fed 2.5 kg day^{-1} of a commercial diet providing 13MJ DE kg^{-1}, and the pens were cleaned as required with straw being provided for bedding twice a week. After a four week period a boar was introduced to the pen daily and was used to serve the gilts on two consecutive days. The expected parturition day (EPD) was calculated as 114 days after the first service date. Once pregnancy was confirmed at around 32 days after service the gilts were housed in groups in a strawed yard (9.6 x 6m) where they were floor fed 2.5 kg day^{-1} of the same commercial diet. Similarly the pens were cleaned and straw for bedding provided twice a week.

5.3.2. Catheterization

All gilts had a jugular vein catheter (silastic, Osteotec Ltd., Christchurch, Dorset, U.K., internal diameter - 1.47 mm and external diameter - 1.93 mm) implanted under general anaesthesia around 15 days before the EPD (15 ± 0.66 days) (for full details of the procedure see Lawrence et al, 1992). Briefly the catheter was protected with an adhesive neck bandage, and a connecting tap at the back of the neck was used for the removal of blood. The catheters were flushed daily with saline and primed with heparinised saline (150 I.U. ml^{-1}). After the operation, the gilts were housed individually in straw-bedded pens (2 m x 2 m). The same commercial feed as before was offered in 2 meals at 0800 and 1600 hrs (2.5 kg day^{-1}). One gilt was removed from the study due to a blocked catheter.

5.3.3. Experimental Housing

Five days before EPD the gilts were weighed and moved to either a conventional farrowing crate (Treatment C (n=15) - 2.25 m in length, 0.45 m in width and 1.05 m in height) or to a pen (Treatment P (n=16) - 2.5m x 3.0m). The crate consisted of a solid floor with slatted dunging area at the back and no straw was provided. The pen had a solid floor which was sloped to allow drainage and straw was provided. Both environments had a creep area outside the specified dimensions. The temperature of the housing was controlled (mean minimum temperature (°C) ± s.e.m. = 15.16 ± 0.05; mean maximum temperature (°C) = 18.24 ± 0.05), and the lights were on between 0800 and 1600 hrs. Dim lights were used to allow observation during the night. The gilts were offered 3kg day^{-1} of a food that provided 13.75MJ DE kg^{-1} and 18% protein in 2 meals at 0800 and 1600 hrs. After parturition the food level was gradually increased to appetite. The pens were cleaned and fresh straw provided after the morning feed, and the slatted area in the crates was also cleaned at this time.

5.3.4. Blood Sampling

Sampling began at the onset of nest-building behaviour (approximately 12 hours pre-farrowing) to obtain baseline levels prior to parturition. Samples were collected through a catheter extension which minimised the disturbance to the gilts by allowing samples to be taken from outside the crate or pen area. The blood sampling protocol can be seen in Table 1. The frequency of sampling increased as parturition approached and at 7.5 minutes after the birth of the first piglet (BFP) the gilts either received naloxone (1mg kg^{-1} body weight: Sigma Aldrich Company Ltd., Gillingham, Dorset, U.K.) or a control dose of saline administered via the jugular vein catheter.

Saline was used to replace the volume of blood taken following each sample and the catheter and extension primed with heparinised saline (75 I.U. ml^{-1}). Heparinised monovette tubes (Sarstedt, Leicester, UK.) were used to collect blood samples of either 7 or 10 ml volume. The samples were kept at 4°C for 30 minutes before

centrifuging. They were then spun at 3000 r.p.m. at 4°C for 20 minutes. Aliquots of plasma were pipetted and stored at -20°C for future assay.

Table 1. Frequency of blood sampling and time periods used in the analysis from 6 hours pre-farrowing to 48 hours post-farrowing.

Period	**Time relative to BFP**	**Sampling Frequency**
Baseline	-6 / -1 hours	30 mins
Hour pre-farrowing	-50 / +5 mins	10 mins
Hour 1	0 / +55 mins	5 mins
Hour 2	+1 / + 1 hour 50 mins	10 mins
Hours 3 and 4	+2 / +4 hours	15 mins
Post-farrowing	+ 48 hours	3 times daily (0800, 1200, 1600)

5.3.5. Radioimmunoassays

Blood samples from different groups and treatments were balanced across assay runs. Plasma used for all assays had been thawed once only.

5.3.5.1. Cortisol

Cortisol was extracted using diethyl ether from 100µl of plasma and concentrations were measured using radioimmunoassay of the extracted steroid (Duncan et al., 1990). The intra and interassay coefficients of variation were 11.25% and 14.96% respectively, and the minimum detectable level of the assay was 0.23 ng ml^{-1}. Single samples were extracted and then assayed in duplicate.

5.3.5.2. β-endorphin

200µl aliquots of plasma were assayed using radioimmunoassay in duplicate. Dextran-charcoal was used in the separation process and the supernatant counted using a multigamma counter (Ebling and Lincoln, 1987). Intra and interassay coefficients of variation were 15.04% and 12.73% respectively, and the minimum detectable level of the assay was 49 pg ml^{-1}.

5.3.6. Statistical Analysis

5.3.6.1. Progress of parturition and piglet information

A two sample t-test (Minitab, version 7.2) was used to determine any differences in gestation length or gilt weight between the two environments. A one way analysis of variance (Minitab, version 7.2) was used to determine differences between the treatment groups in length of parturition and piglet interval, the number of piglets born alive and the litter and mean piglet weight. To determine differences between environment and injection groups in the time of day at BFP, the number of gilts which savaged, crushed or had still born piglets, a Fisher's Exact Test or Chi-Square test was carried out.

5.3.6.2. Hormonal analysis

The results of the above assays were divided into time periods (Table 1). A two sample t-test (Minitab, version 7.2) was used to determine if there were any differences between the treatment groups in the baseline period. The data was then normalised by $\log^{10}$ transformation and analysed using a repeated measures analysis of variance (ANOVA; Genstat version 5), with a blocked structure for pig and time period. Factors were environment (two levels; crate (C) and pen (P)), injection (two levels; naloxone (N) or saline (S)), treatment (four levels; P/N, P/S, C/N, C/S), group (eight levels) and time period (six levels).

To look more closely at changes within each time period a repeated measures analysis of variance was carried out on individual samples during each of the time periods. Therefore analysis was carried out for the hour pre-farrowing, hour 1, hour 2 and hours 3 and 4, and then these four time periods combined. The sample taken at 5 minutes after the BFP was used as a baseline for the injection and was therefore removed from subsequent concentrations during the four hours after the birth of the first piglet. Results are given as the variance ratio (F) with subscripts indicating degrees of freedom. Statistical significance was accepted at $p<0.05$, however some tendencies ($p<0.10$) are presented.

Post-hoc analysis of effects of environment and injection involved the use of two-sample t-tests and Mann Whitney tests (Minitab, version 7.2) and treatment effects involved the use of a Least Significant Difference (LSD) test (Genstat, version 5).

5.4. Results

5.4.1. Progress of parturition and piglet information

There was no difference in the length of gestation between the gilts in the two environments (mean length of gestation (days) ± s.e. = 113.47 ± 0.30 and 113.44 ± 0.33 for crate and pen gilts respectively) or in the time of day at which the first piglet was born ($\chi^2 = 1.4$, n.s.). There was also no difference between the weight of the gilts on entrance to the farrowing accommodation (mean gilt weight (kg) ± s.e. = 200.2 ± 3.96 and 200.6 ± 3.01 for crate and pen gilts respectively). The length of parturition was not affected by treatment (mean parturition length (hours) = 3.82 (C/N), 4.59 (C/S), 3.10 (P/N), 2.67 (P/S), s.e.d. = 0.74). Therefore piglet interval was also not affected by treatment (mean piglet interval (mins) = 22.22 (C/N), 23.39 (C/S), 13.6 (P/N), 15.5 (P/S), s.e.d. = 4.31). The number of piglets born alive and the mean piglet weight was not affected by treatment with the mean litter size being 11.78 ± 0.51 and the mean piglet weight (kg) being 1.3 ± 0.04.

5.4.2. Piglet Deaths

There was a tendency for a greater number of gilts in crates to give birth to dead (not mummified) piglets than gilts in pens ($p = 0.075$). There was no difference between the number of gilts involved in savaging or crushing piglets, however the differences in the actual number of piglets that were killed due to savaging or crushing (Table 2) suggest large individual differences, with, for example, certain gilts crushing many piglets.

Table 2. Information on piglet deaths including stillborn, savaged and crushed piglets only between the two environments.

	Crate	**Pen**
Number of gilts	15	16
Total piglets born	185	206
Piglets born dead (% of total)	7 (3.8)	2 (0.97)
Piglets savaged (% of total)	5 (2.7)	1 (0.49)
Piglets crushed (% of total)	7 (3.8)	28 (13.6)
Total loss (% of total)	19 (10.3)	31 (15.0)

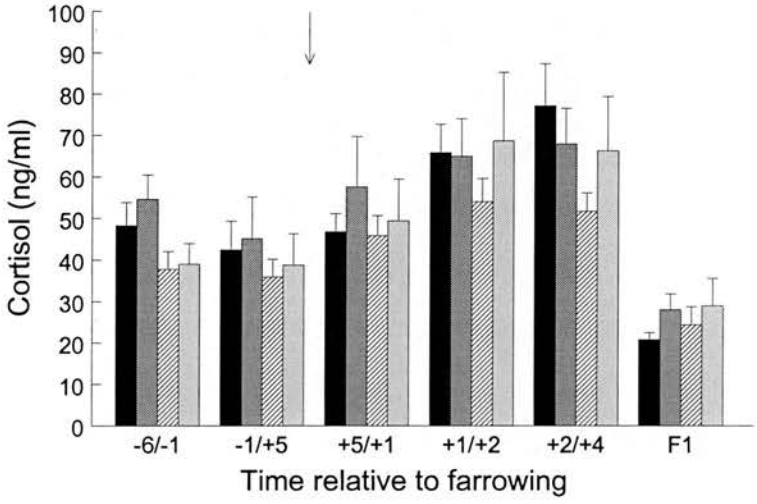
5.4.3. Cortisol

As there was no difference between the treatment groups in the baseline period ($F_{3,29}$ = 2.22 p = 0.109) the remaining time periods were not adjusted to account for the baseline. From the time period analysis an effect of time was found ($F_{5,133}$ = 49.91, p<0.001) with plasma cortisol concentrations gradually increasing in all treatments from the pre-farrowing period over the 4 hours following the BFP and then falling by 48 hours post-farrowing (Figure 1a).

Using individual samples to examine the time periods in greater detail, it was found that in the hour pre-farrowing there was a tendency for crated gilts to have higher concentrations of plasma cortisol than penned gilts (mean cortisol concentration (ng/ml) ± s.e. = 46.4 ± 2.87 and 38.9 ± 2.4 for crate and pen gilts respectively; time x environment interaction: $F_{6,138}$ = 1.89, p=0.087).

During the first hour (0-60 minutes) following BFP, cortisol concentrations gradually increased (Time Effect: $F_{9,232}$ = 2.80, p<0.01; Figure 2c). There was no effect of environment (Figure 2a) or naloxone (Figure 2b) on cortisol concentration at this time. Similarly during the second hour (60-120 minutes) plasma cortisol concentrations continued to increase (Time Effect: $F_{5,128}$ = 2.03, p=0.078; Figure 2c). This increase in cortisol continued during Hours 3 and 4 (120-240 minutes) (Time Effect: $F_{8,183}$ = 4.92, p<0.001), however C/N gilts had higher concentrations of cortisol than the other treatments at this time (time x treatment interaction: $F_{24,183}$ = 1.93, p<0.01; Figure 2c).

a)



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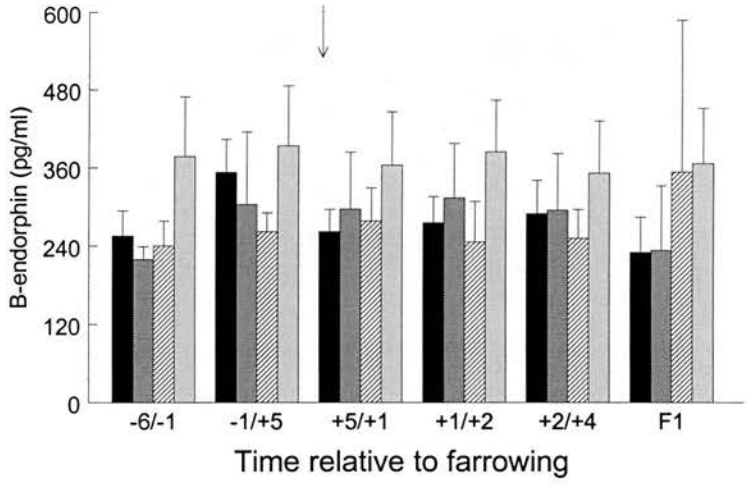
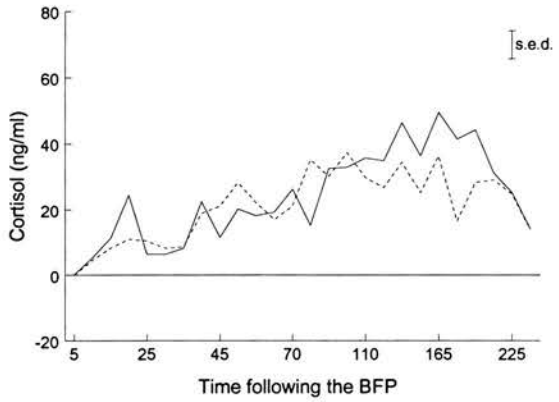


Figure 1. Concentrations of plasma a) cortisol and b) β-endorphin over the designated time periods for the 4 treatment groups: C/N (■, n=8), C/S (▒, n=7), P/N (▨, n=8), P/S (□, n=8). F1 = 48 hours after the birth of the first piglet. Arrow indicates administration of naloxone or saline.

a)



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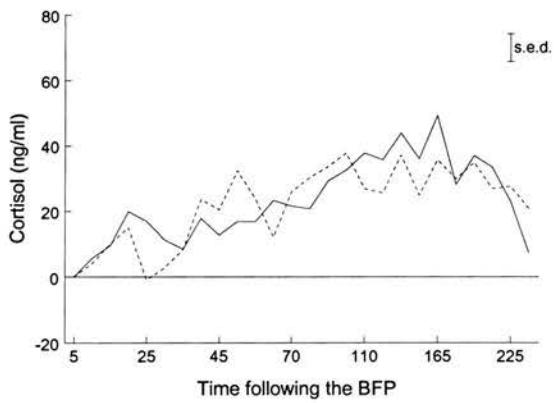


c)

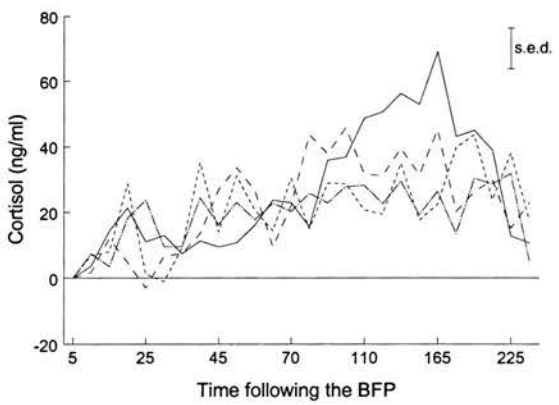


Figure 2. Concentrations of plasma cortisol during the 4 hours following the birth of the first piglet (BFP) presented in terms of a) environment; Crate (—, n=15) and Pen (---, n=16), b) injection; Naloxone (—, n=16) and Saline (---, n=15) and c) treatment; C/N (—, n=8), C/S (---, n=7), P/N (-·-, n=8) and P/S (····, n=8). The sample taken at 5 minutes post-BFP is used as a baseline and is therefore removed from subsequent samples.

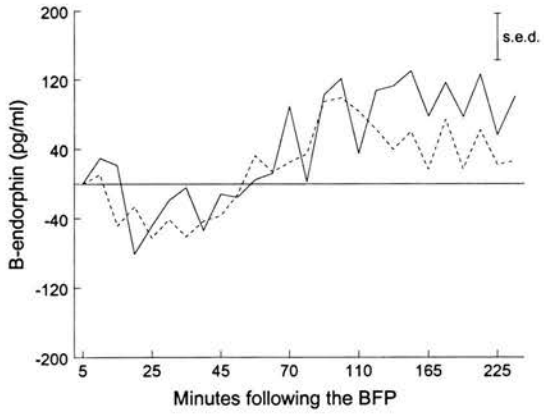
5.4.4. β-endorphin

As there was no difference between the treatment groups in the baseline period ($F_{3,29}$ = 1.32, p=0.288) the remaining time periods were not adjusted to account for the baseline. Analysis of the data by time periods (Figure 1b) found that penned gilts had higher concentrations of β-endorphin 48 hours after farrowing (Time x treatment interaction: $F_{15,101} = 4.13$, $p<0.001$; Figure 1b).

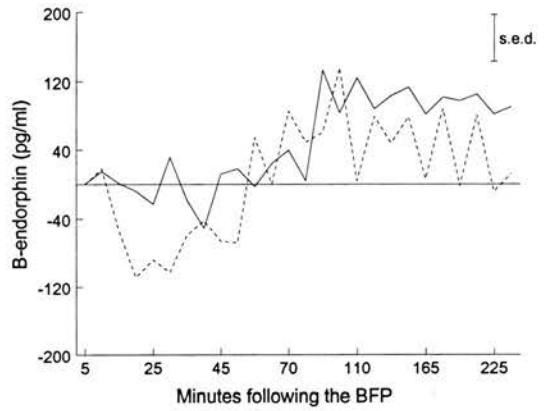
Plasma β-endorphin concentrations changed with time during the first hour following BFP ($F_{9,84} = 9.81$, $p<0.001$). Generally crated gilts had higher levels of plasma β-endorphin during the first hour post-BFP however this was at a non-significant level. However a decrease in plasma β-endorphin of the crated gilts below that of the penned gilts at 20 minutes post-BFP resulted in a time x treatment interaction ($F_{9,101}$ = 2.36, $p<0.05$) (Figure 3a). In general the saline treated gilts in both environments showed a fall in β-endorphin following BFP, whilst naloxone treated gilts had fairly constant concentrations of plasma β-endorphin during this period (Time x injection interaction: $F_{9,101} = 7.90$, $p<0.001$; Figure 3b).

Plasma β-endorphin concentrations increased during the second hour (60-120 minutes) following BFP (Time; $F_{5,67} = 3.00$, $p<0.01$; Figure 3c), however there was no effect of time, environment (Figure 3a) or injection (Figure 3b) during the third and fourth hour (120-240 minutes) after the onset of parturition.

a)



b)



c)

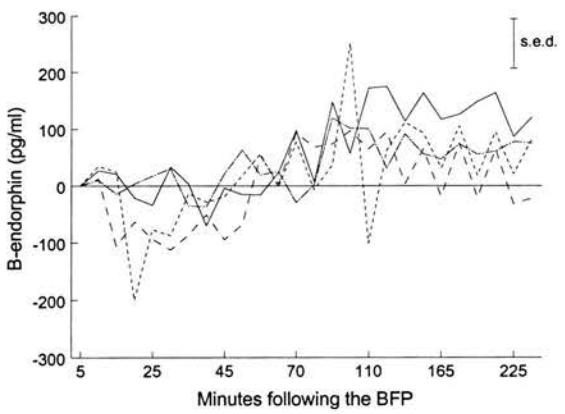


Figure 3. Concentrations of plasma β-endorphin during the 4 hours following the birth of the first piglet (BFP) presented in terms of a) environment; Crate (—, n=15) and Pen (---, n=16), b) injection; Naloxone (—, n=16) and Saline (---, n=15) and c) treatment; C/N (—, n=8), C/S (---, n=7), P/N (-·-, n=8) and P/S (····, n=8). The sample taken at 5 minutes post-BFP is used as a baseline and is therefore removed from subsequent samples.

5.5. Discussion

This study has shown that changes in plasma cortisol and β-endorphin during farrowing are not affected by environment. It has been shown previously that crated gilts have higher levels of plasma cortisol in the active phase of the pre-farrowing period (Jarvis et al., 1997b) which is consistent with the present study in which crated gilts had higher cortisol in the hour before the birth of the first piglet. However the difference between the two environments was not as apparent as that seen in the study by Jarvis et al., 1997b, which most likely reflects the reduction in activity that occurs in the few hours before birth (Jarvis et al., 1997b, Jensen, 1996) and therefore the lessening of the restriction imposed by the crate at this time.

β-endorphin levels generally increased during the first two hours following the birth of the first piglet, then remained constant over the remainder of the farrowing period and continued to be high 48 hours after the birth of the first piglet. However naloxone appeared to prevent the fall in β-endorphin in the first hour following BFP seen in the saline treated gilts, which perhaps reflects the immediate release of opioids from the anterior pituitary into the circulation. β-endorphin is known to increase in women at parturition (Fettes et al., 1984, Fajardo et al., 1994) and there is evidence to suggest that there is an inverse relationship between plasma β-endorphin and pain perception (Raisanen et al., 1984, Varrassi et al., 1989). Jarvis et al., 1997b, have shown an increase in plasma β-endorphin from 5 days pre-farrowing, continuing until the birth of the first piglet. Opioid-mediated analgesia occurs in the pig prior to parturition (Jarvis et al., 1997a), and this elevation in plasma β-endorphin may be related to the onset of this analgesia through its action at peripheral sites (Joris et al., 1987). Further increases in plasma β-endorphin following the birth of the first piglet may result from the pain and novelty associated with expulsion. The lack of further rises in plasma β-endorphin in the later stages of farrowing may be a result of the reduced number of piglet births during this time.

The role of the foetal HPA axis in the initiation of parturition has been demonstrated in the sheep (Liggins et al., 1969), however the role of the piglet is not clear as a study by Randall et al., (1990) has shown that the infusion of piglets, *in utero*, with ACTH does not cause premature delivery. However the contribution of foetal cortisol to maternal plasma cortisol may cause differences between gilts, however due to the lack of difference between environments in terms of litter size, length of parturition and piglet birth interval any foetal contribution is balanced across the environments.

The continued increase in cortisol seen over the farrowing period in this study was also seen in a study by Lawrence et al., 1994, and consistent with this study decreased over the days post-farrowing. As the changes in cortisol concentration seen in this study were insensitive to environment this suggests that certain aspects of parturition itself stimulate the HPA axis. Potential factors may be cumulative piglet births as a result of the pain or novelty experienced due to the process, or the interaction with piglets with which the gilt has no prior experience. This is consistent with a study by Jarvis et al., in press b, in which plasma cortisol concentration following the birth of a piglet increased with piglet number. As farrowing progresses the number of piglets which are suckling at the udder increases and this stimulus may be involved in increasing plasma cortisol. There is evidence to suggest that suckling causes a rise in plasma cortisol in the rat (Walker et al., 1992) following a separation period. However it is unknown whether suckling-induced cortisol release is a result of some stress-inducing effect of suckling or whether there is a functional metabolic effect of this release.

It appears overall that there is limited opioid control of the HPA axis in parturient pigs. This is in contrast to work in young non-pregnant female pigs in which naloxone increased plasma cortisol and ACTH concentrations in response to nose restraint (Rushen and Ladewig, 1991, Rushen et al., 1993). However in a more recent study by Rushen et al., (1995), it was found that the reduced HPA response in

lactating pigs is not a result of increased opioid inhibition as cortisol concentrations in response to nose restraint were not elevated following naloxone. This appears to reflect some change in the sensitivity of the negative feedback control of the HPA axis in pregnancy and lactation to opioid restraint. Work has shown that cyclic rats have elevated ACTH and cortisol concentrations during the pro-oestrus phase when oestrogen levels are high (Carey et al., 1995). Ovariectomised rats treated with oestrogen only or oestrogen and progesterone showed an increase in plasma ACTH and cortisol; in contrast progesterone alone had no effect on HPA responsiveness (Carey et al., 1995). It is known that the increase in plasma cortisol seen in late pregnancy is not due to an increase in cortisol binding globulin (Scott et al., 1990), and therefore perhaps oestrogen, which rises from around 80 days of pregnancy in the pig (Robertson and King, 1974, Robertson et al., 1985) may be involved in resetting the sensitivity of the HPA axis during pregnancy (Nolten et al., 1980).

In conclusion this study provides little evidence of opioid control of the HPA axis at parturition and suggests that environment has little effect on the release of β-endorphin or cortisol during farrowing. This may reflect the decreased activity of the sow at this time therefore lessening the effect of the behavioural restriction imposed by the crate. The rise seen in plasma β-endorphin and cortisol over the farrowing period appears to be a response to certain aspect(s) of parturition itself. However it remains to be determined whether this 'birth stress' reflects a normal metabolic function during parturition and early lactation, or is a response by the sow to the painful and novel aspects to which she is inevitably exposed during delivery.

Chapter 6

The effect of piglet expulsion in the sow on plasma cortisol, ACTH and β-endorphin

6.1. Abstract

Previous studies have shown an increase in plasma cortisol in gilts over farrowing irrespective of environment suggesting that factor(s) associated with parturition itself cause physiological stress. Factors involved in mediating the HPA axis at parturition are not well understood. This study examines the effect of piglet expulsion on the pituitary-adrenal axis by measurement of plasma cortisol, ACTH and β-endorphin. The effect of farrowing environment in modulating the acute response to piglet expulsion is also investigated. Twelve second parity sows, with indwelling jugular catheters, were moved into either a farrowing crate or a straw-bedded pen 5 days before their expected parturition date (EPD). Blood samples were taken from each sow during a pre-farrowing baseline period and then rapid samples (2.5 mins) were taken for 10 minutes following the birth of two piglets. No effect of environment was found on any of the hormonal variables which reinforces the hypothesis that the physiological stress seen in parturient pigs is due to some intrinsic factor of parturition. Plasma cortisol, ACTH and β-endorphin did not change significantly in the period following piglet expulsion suggesting that individual piglet expulsions do not play a major role in 'parturition stress'. There was however an increase in plasma cortisol, ACTH and β-endorphin in response to increasing piglet number which is consistent with previous studies of general farrowing in which cortisol increased as farrowing progressed. Therefore this study reinforces the hypothesis that physiological stress increases with ongoing parturition although this does not appear to be a result of piglet expulsion. The potential role of other factors which may be involved in causing 'parturition stress' should be investigated.

6.2. Introduction

Previous work has shown that in the pig plasma cortisol concentration continuously increases during the delivery phase of farrowing and then falls within one day post-farrowing (Lawrence et al., 1994, Jarvis et al., in press a), suggesting that parturition causes physiological stress in the pig. This physiological stress is not due to environmental restriction as this rise in plasma cortisol is seen in both gilts housed in conventional farrowing crates and straw-bedded pens (Lawrence et al., 1994, Jarvis et al., in press a). Factor(s) associated with parturition such as uterine contraction, pain and novelty of piglet expulsion, and the interaction with novel piglets may contribute to this 'parturition stress' which is characterised by increased HPA activity.

We decided to examine the effect of piglet expulsion in this experiment as the pain associated with giving birth, and the novelty associated with this process may be involved in the increased HPA activity seen during delivery. We chose to measure plasma cortisol and ACTH which are commonly used to indicate physiological stress around parturition (Kaupilla et al., 1974, Fettes et al., 1984, Lawrence et al., 1994, Jarvis et al.,1997b) and there is a significant correlation between cortisol and anxiety in women during labour (Lederman et al., 1978). Therefore measurement of these two hormones allows examination of the pituitary-adrenal axis during farrowing. In addition, evidence suggests that in pregnant women there is an inverse relationship between plasma β-endorphin and pain perception (Raisanen et al., 1984, Varrassi et al., 1989). Therefore β-endorphin, an endogenous opioid released from the anterior pituitary (Guillemin et al., 1977), may be involved in producing analgesia at peripheral sites (Joris et al., 1987) and therefore circulating plasma concentrations may indicate levels of nociception resulting from piglet expulsion.

Therefore the objective of this study was to examine the potential role of piglet expulsion in 'parturition stress'. By measurement of plasma cortisol, ACTH and β-endorphin we could determine the effect of piglet expulsion on pituitary and adrenal

release. Although in previous work no environmental effect was found in terms of plasma cortisol and β-endorphin over the farrowing period (Jarvis et al., in press a), we investigated, in the present study, the more acute effects of piglet expulsion in both crated and penned sows.

6.3. Materials and Methods

6.3.1. Animals

The subjects of this study were 12 Large White x Landrace second parity sows which had been previously been acquired as primiparous sows (gilts; Cotswold Pig Development Co. Lincoln, UK). The sows were group housed in straw-bedded pens (2.6 m x 4.1 m) which were cleaned as required with straw being provided for bedding twice a week. Water was available *ad libitum* and the sows were fed 2.5 kg day^{-1} of a commercial diet providing 13 MJ DE kg^{-1}. A boar was introduced to the pen daily and the sows were served on two consecutive days. The expected parturition day (EPD) was calculated as 114 days after the first service date. Once pregnancy was confirmed at around 32 days after service the sows were housed in groups in a strawed yard (9.6m x 6m) where they were floor fed 2.5 kg day^{-1} of the same commercial diet. Similarly the pens were cleaned and straw for bedding provided twice a week.

6.3.2. Catheterization

All sows had a jugular catheter (silastic, Osteotec Ltd., Christchurch, Dorset, U.K., internal diameter - 1.47 mm and external diameter - 1.93 mm) implanted under general anaesthesia around 13 days before the EPD (12.67 ± 0.27 days) (for full details of the procedure see Lawrence et al, 1992). Briefly, the catheter was protected with an adhesive neck bandage, and a connecting tap at the back of the neck was used for the removal of blood. The catheters were flushed daily with saline and primed with heparinised saline (150 I.U. ml^{-1}) until sampling began 10 days before EPD. After the operation, the sows were housed individually in straw-bedded pens (2 m x 2 m). The same commercial feed was offered in 2 meals at 0800 and 1600 hrs (2.5 kg day^{-1}).

6.3.3. Experimental Housing

Five days before EPD the sows were weighed and moved to either a conventional farrowing crate (Treatment C (n=6) - 2.25 m in length, 0.45 m in width and 1.05 m in height) or to a pen (Treatment P (n=6) - 2.5m x 3.0m). The experimental housing for each sow was the same as that used for their first farrowing and lactation. The crate consisted of a solid floor with slatted dunging area at the back and no straw was provided. The pen had a solid floor which was sloped to allow drainage and straw was provided. Both environments had a creep area outside the specified dimensions. The temperature of the housing was controlled (mean minimum temperature (°C) ± s.e.m. = 16.42 ± 0.08, mean maximum temperature (°C) = 19.54 ± 0.13), and the lights were on between 0800 and 1600 hrs. Dim lights were used to allow observation during the night. The sows were offered 3kg day^{-1} of a food that provided 13.75 MJ DE kg^{-1} and contained 18% protein in 2 meals at 0800 and 1600 hrs. After parturition the food level was gradually increased to appetite. The pens were cleaned and fresh straw provided after the morning feed, and the slatted area in the crates were also cleaned at this time.

6.3.4. Blood Sampling

Samples were collected into heparinised monovette tubes (Sarstedt, Leicester, UK.) through a catheter extension which minimised the disturbance to the sows by allowing samples to be taken from outside the crate or pen area. Blood samples were taken every 30 minutes during the 2 hours before the onset of parturition to obtain baseline concentrations (Figure 1). Once 30 minutes had passed since the onset of parturition and at least two piglets had been born, a window of blood samples was taken following the birth of the next piglet. This consisted of taking a sample at the birth of the piglet and then every 2.5 minutes until 10 minutes had passed i.e. 5 samples. A second window of samples was taken following the birth of another piglet allowing one piglet to be born between the windows (Figure 1). A window was not carried out following multiple births. Piglets were not weighed at birth to avoid disturbing the

gilt, however were categorised as small (S), medium (M) or large (L) and weighed the following day. A pilot study on non-test pigs was carried out to determine the weight range of each of the categories to estimate weight at birth. Saline was used to replace the volume of blood taken following each sample and the catheter and extension primed with heparinised saline (75 I.U. ml^{-1}). The samples were kept at 4°C before centrifuging at 3000 r.p.m. for 20 minutes. Aliquots of plasma were pipetted and stored at -20°C for future assay.

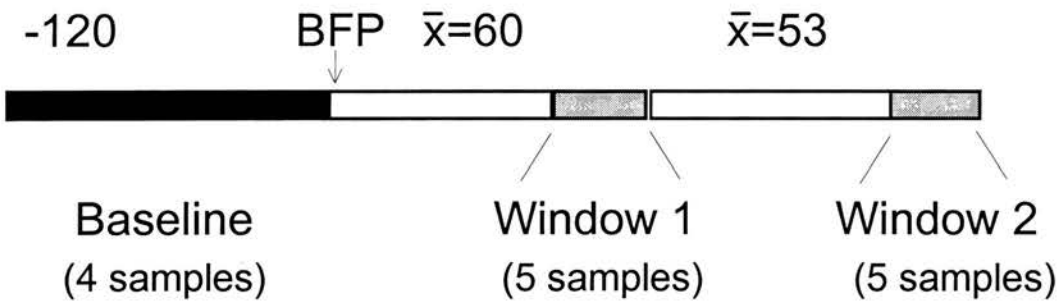


Figure 1 Blood sampling procedure; numbers indicate minutes around the birth of the first piglet (BFP) with the mean number of minutes separating windows indicated. The baseline period (sample every 30 minutes) and the two windows are shown, with the two windows being separated by the birth of at least one piglet. A window includes a sample at the birth of a piglet and then every 2.5 minutes for 10 minutes (5 samples).

6.3.5. Radioimmunoassays

6.3.5.1. Cortisol

Cortisol was extracted using diethyl ether from 100µl of plasma and concentrations were measured using radioimmunoassay of the extracted steroid (Duncan et al., 1990). The intra and interassay coefficients of variation were 12.8% and 10.9% respectively, and the minimum detectable level of the assay was 0.16 ng ml^{-1}. Samples were extracted and assayed in duplicate.

6.3.5.2. ACTH

ACTH concentrations were measured in single aliquots of 100µl of plasma using an immunoradiometric assay kit (Euro Path Ltd., Bude, Cornwall, UK). Second antibody method was used, the samples spun and the pellet counted using a multigamma counter (Brooks, 1992). The minimum detectable level of the assay was 10 pg ml^{-1} and the intra and inter-assay coefficients of variation was 11.2% and 21.8%.

6.3.5.3. β-endorphin

200µl aliquots of plasma were assayed using radioimmunoassay in duplicate. Dextran-charcoal was used in the separation process and the supernatant counted using a multigamma counter (Ebling and Lincoln, 1987). Intra and interassay coefficients of variation were 15.5% and 12.25% respectively, and the minimum detectable level of the assay was 195 pg ml^{-1}.

6.3.6. Statistical analysis

6.3.6.1. Parturition and Piglet Information

A two sample t-test (Minitab, version 7.2) was used to determine any differences in length of gestation and parturition between the two environments. Differences in piglet interval, the number of piglets born alive and the litter and mean piglet weight were also investigated.

6.3.6.2. Hormonal Measures

A two-sample t-test (Minitab, version 7.2) was used to determine if there was any difference between the environments in the baseline period for each of the three hormones. Analysis of the samples taken in the two windows was carried out using a repeated measures analysis of variance (Genstat, version 5) blocked for pig and window, with factors pig (12 levels), treatment (2 levels), window (2 levels) and sample (10 levels). Piglet size and number, time since the last piglet was born and time since the onset of parturition were used as covariates. Post-hoc analyses of any significant effects were carried out using two-sample t-tests and mann whitney tests (Minitab, version 7.2) depending on the distribution of the data.

Results are presented as variance ratios (F) and t-statistics (t) with subscripts referring to the number of degrees of freedom. Statistical significance was accepted at $p<0.05$ however some tendencies are presented.

6.4. Results

6.4.1. Parturition and Piglet Information

There was no difference between the length of gestation of the sows in the two environments (mean gestation length (days) ± s.e.m. = 114.3 ± 0.56 and 115.0 ± 0.37 for crate and pen sows respectively). Parturition length also did not differ between the environments (mean length of parturition (hours) ± s.e.m. = 4.31 ± 0.82 and 3.96 ± 0.35 for crate and pen sows respectively). There was also no difference in the number of piglets born (11.67 ± 1.26 and 12 ± 1.13 for crate and pen sows) and the mean piglet weight at 24 hours old (1.63 ± 0.14 and 1.61 ± 0.14 for crate and pen sows). More piglets were born dead in crates, however there was a greater incidence of crushing in the pens (Table 1). Regarding the sampling windows, there was no difference between environments in terms of piglet number at each window (Mean piglet number ± s.e. = 3.5 ± 0.3 and 3.8 ± 0.3 for crate and pen sows respectively for Window 1 ($p=0.60$), and 5.7 ± 0.3 and 6.3 ± 0.3 for crate and pen sows for Window 2 ($p=0.19$)). Similarly there was no difference between the environments regarding the time since the onset of farrowing for each window (Mean time since start of farrowing (minutes) ± s.e. = 69.6 ± 19.2 and 51.6 ± 6.6 for crate and pen sows respectively for Window 1 ($p=0.42$), and 121.2 ± 27.6 and 110.4 ± 15.0 for crate and pen sows for Window 2 ($p=0.74$)). From the pilot study the weight ranges of different size piglets were: Small (0.75-1.25 kg), Medium (1.25-1.72 kg), Large (1.72-2.46 kg).

Table 1 Information on piglet deaths including stillborn, savaged and crushed piglets only between the two environments.

	Crate	**Pen**
Number of sows	6	6
Total piglets born	70	72
Piglets born dead (% of total)	5 (6.7)	1 (1.4)
Piglets savaged (% of total)	1 (1.4)	0 (0.0)
Piglets crushed (% of total)	5 (7.1)	16 (22.2)
Total loss (% of total)	11 (15.2)	17 (23.6)

6.4.2. Cortisol

There was no significant difference between the plasma cortisol concentrations of the sows during the baseline period with regard to environment, therefore subsequent concentrations were not adjusted to account for the baseline (mean plasma cortisol concentration ± s.e. = 41.3 ±7.2 and 35.9 ± 5.2 for crate and pen sows respectively, t_9 = 0.60, p = 0.56). In the period following the birth of a piglet plasma cortisol was not affected by the environment ($F_{1,6}$ = 0.32, p=0.59) or time ($F_{1,6}$ = 2.20, p=0.19, Figure 2a). During window 2 plasma cortisol concentrations remained high over the entire 10 minute period (Figure 2b). There was a tendency for piglet number to affect plasma cortisol ($F_{1,6}$ = 3.94, p=0.095) with increased cortisol being seen following the birth of later piglets, in particular piglets 5 and 7. Plasma cortisol following the birth of a piglet was also affected by the size of the specific piglet $F_{1,6}$ = 4.92, p=0.068) with the sample taken immediately following the birth of a large piglet being significantly greater than for a small or medium piglet (Figure 3) (S-L; t_{12} = -2.96, p=0.012, M-L; t_{12} = -3.77, p<0.01)

6.4.3. ACTH

There was no significant difference between the plasma ACTH concentrations of the sows during the baseline period with regard to environment, therefore subsequent concentrations were not adjusted to account for the baseline (mean plasma ACTH concentration ± s.e. = 32.5 ± 7.87 and 46.0 ± 23.8 for crate and pen sows respectively, t_6 = -0.54, p = 0.61). Plasma ACTH concentrations also did not change in the period following the birth of piglets ($F_{1,6}$ = 0.04, p=0.843) at Window 1 (Figure 2c) or Window 2 (Figure 2d) and were not affected by the farrowing environment ($F_{1,6}$ = 0.79, p=0.408). The number of piglet at each of the windows affected plasma ACTH concentrations ($F_{1,68}$ = 3.22, p = 0.077), with ACTH levels increasing with piglet number. Similarly to cortisol, piglet 5 and piglet 7 resulted in the greatest response in terms of plasma ACTH concentration.

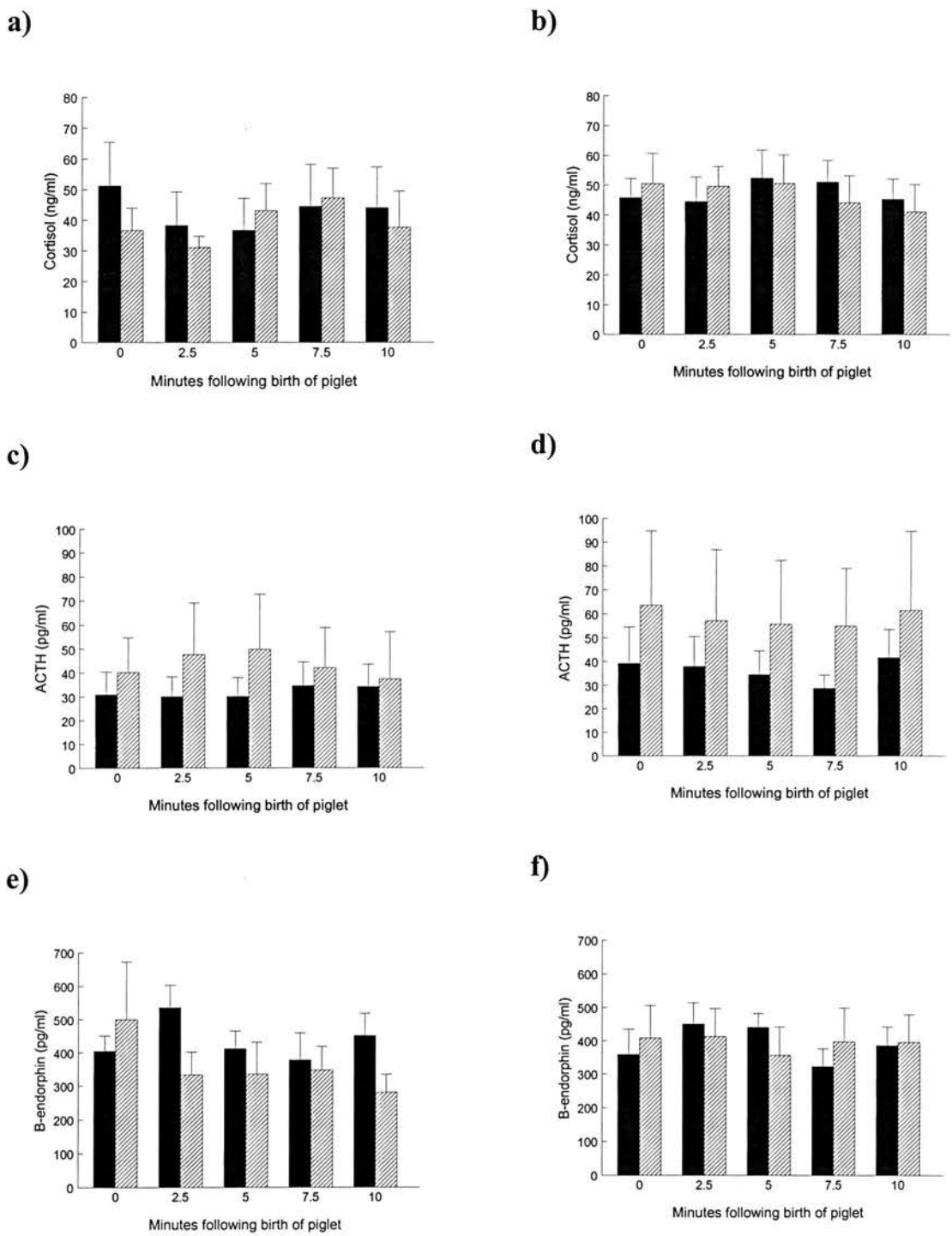


Figure 2 Plasma concentrations; Cortisol during a) Window 1 and b) Window 2, ACTH during c) Window 1 and d) Window 2, β-endorphin during e) Window 1 and f) Window 2 for crated (■, n=6) and penned (▨, n=6) sows.

6.4.4. β-endorphin

There was no significant difference between the plasma β-endorphin concentrations of the sows during the baseline period with regard to environment, therefore subsequent concentrations were not adjusted to account for the baseline (mean plasma β-endorphin concentration ± s.e. = 424 ± 77.3 and 286 ± 59.3 for crate and pen sows respectively, t_9= 1.42, p = 0.19). At the birth of piglets, there was no significant difference in plasma β-endorphin between the environments ($F_{1,6}$ = 0.29, p=0.61; Figure 2e and 2f). There was also no change over time following the birth of a piglet ($F_{1,6}$ = 0.08, p=0.787). Plasma β-endorphin concentrations were also affected by piglet number ($F_{1,68}$ = 7.44, p<0.01) with levels increasing with piglet number.

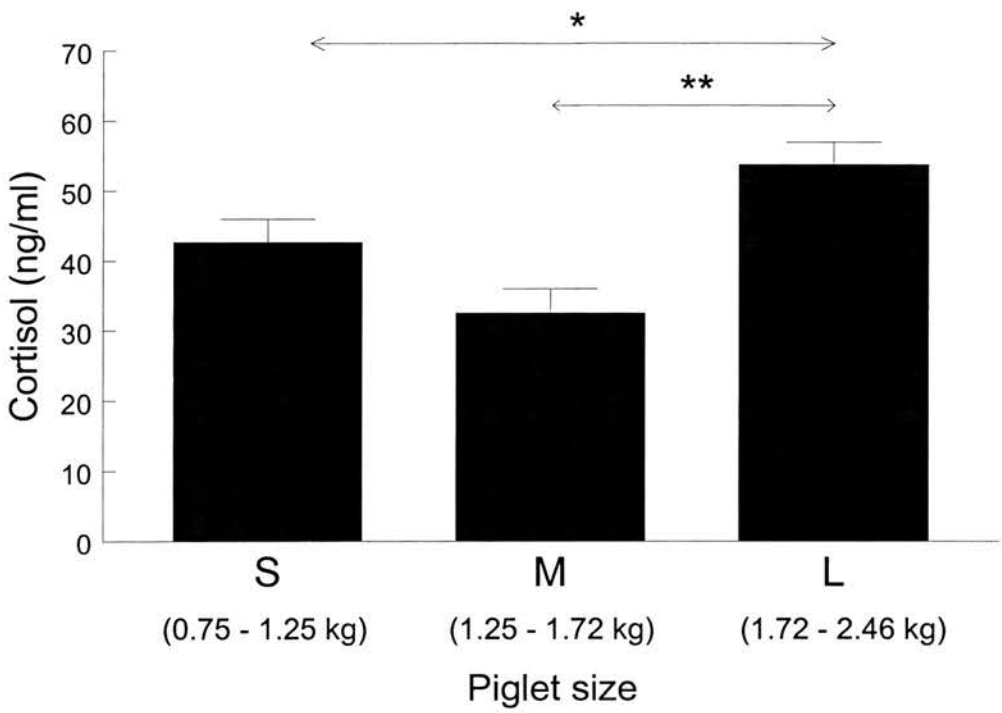


Figure 3. Mean plasma cortisol concentrations of the sample taken at the beginning of every window with piglets categorised according to size (S = small, M = medium, L = large). * $p<0.05$, *** $p<0.001$. Numbers in parenthesis indicate weight range of each size category.

6.5. Discussion

Similar to previous studies examining changes in plasma concentrations of cortisol (Lawrence et al., 1994, Jarvis et al., in press a) and β-endorphin (Jarvis et al., in press a) over the majority of the farrowing period, this study also found no effect of the farrowing accommodation at this time. Therefore the lack of effect of environment seen in previous studies (Lawrence et al., 1994, Jarvis et al., in press a) does not mask any environmental effects on the acute response of the HPA axis to the expulsion of a piglet. Hence this lack of effect of environment in the present study reinforces the hypothesis that factor(s) intrinsic to the parturition process itself are involved in causing physiological stress.

As the plasma concentrations of cortisol, ACTH and β-endorphin did not change in the period following the expulsion of a specific piglet, the role of piglet expulsion in 'parturition stress' appears doubtful. This is consistent with a previous study by Gilbert et al., 1996 in which plasma cortisol concentrations were not related to piglet expulsion. As plasma β-endorphin concentrations did not change in response to piglet birth this suggests that the actual expulsion of a foetus may not be highly associated with pain perception. This may be a result of the opioid-mediated analgesia associated with the entire farrowing period (Jarvis et al., 1997a) with plasma β-endorphin having already reached maximal concentrations to induce analgesia at peripheral sites (Joris et al., 1987). There was however an effect of piglet size on cortisol with larger piglets resulting in a greater immediate cortisol response which suggests that expulsion of a perhaps larger than optimal size piglet may be stress-inducing. From a production perspective, this may suggest that breeding for increased piglet birth weight may cause increased physiological stress at parturition.

The increase in plasma cortisol, ACTH and β-endorphin concentrations with increasing piglet number is consistent with the increase seen over the entire farrowing period in previous studies (Lawrence et al., 1994, Jarvis et al., in press a) and suggests

a cumulative effect of factor(s) associated with parturition over time. This may be due to several potential factors such as the increasing number of piglets with which the mother interacts. There have been no studies to date which deal with the effect on sows of piglets as novel objects during farrowing. However a study by Hutson et al., 1992 found that the reaction time of gilts to distress calls of piglets was shorter than that for older sows suggesting an element of novelty. A second factor which may be involved in 'parturition stress' are the uterine contractions that accompany delivery. Work by Taverne et al., 1979 showed that the frequency of uterine contractions which occur during farrowing is similar to the pre-farrowing period however they are of shorter duration. In a study by Gilbert et al., 1996 plasma cortisol following the birth of piglets was lower than the mean values seen over the entire farrowing period. Therefore events prior to expulsion such as movement of the piglet through the birth canal or uterine contractions may be responsible for the increasing plasma cortisol over farrowing. In the present study there are no samples taken prior to expulsion due to the uncertainty of the exact time of any birth and therefore the effect of uterine contraction and movement of piglets through the birth canal cannot be assessed. Future work should incorporate control samples being taken during the farrowing period prior to expulsion to determine more clearly the effect of movement and expulsion of piglets.

A further potential factor which increases during farrowing is the suckling stimulus. Suckling stimulation is associated with increases in plasma oxytocin (Fuchs et al., 1984), however suckling for 3 hours after a 12 hour separation period has also been shown to increase plasma ACTH and cortisol concentrations (Voogt et al., 1969). In a more recent study in which only a 4 hour separation period was used a similar increase in plasma ACTH and cortisol was seen (Walker et al., 1992). Therefore part of a 'normal' farrowing, and thus suckling, may involve an increase in plasma cortisol and ACTH concentrations. The effect of suckling on plasma cortisol and ACTH during the farrowing period has not been investigated, however work by Stern and

Levine, 1972 has shown that the normal fall in pituitary adrenal responsiveness over lactation is not seen in parturient rats with pups present but following removal of teats, suggesting modification of the pituitary-adrenal axis by the suckling stimulus. Plasma cortisol is known to increase amino acids, free fatty acids and glucose in the blood (Moore, 1984) and therefore the role of cortisol may be to increase these precursors required for further milk production (McDonald et al., 1988).

The behaviour of the sow during farrowing has been described both descriptively (Jones, 1966b and Randall, 1972) and quantitatively (Jarvis et al., unpublished data) with all these studies observing a general inactivity with the sow spending the greatest amount of time in lateral recumbency. Therefore the inability of the sow to perform specific behaviours does not appear to play a major role in the stress associated with the parturition process, however this may be an adaptation to a conventional housing system. Jensen (1986) has observed sows in a free-ranging environment changing posture in the early stages of farrowing however does not discuss the actual proportion of time spent in any particular posture.

In conclusion plasma cortisol, ACTH and β-endorphin concentrations do not change significantly in the period immediately following piglet expulsion suggesting that the actual expulsion of a piglet does not play a major role in 'parturition stress'. However there are factors associated with the parturition process that may act individually or as a combination to cause stimulation of stress-sensitive pathways and should now be investigated.

Chapter 7

General Discussion

7.1 Introduction

The aim of the present thesis was to advance our understanding of parturition in the pig in terms of stress associated with this process and the effects of environmental restriction. This has involved a wide range of disciplines including physiology, neuroscience and behaviour enabling me to examine parturition in the pig from a broader perspective.

In the preceding chapters the behaviour of the pig around farrowing has been quantitatively described and the involvement of opioids at this time has been examined. In addition the effect of environmental restriction on both the behaviour and physiology of the pig has been investigated suggesting potential welfare implications during the pre-parturient phase.

In the following discussion I will describe the behavioural and physiological changes which occur in the pig across the different phases of parturition. In addition I will discuss the current results in an evolutionary context, and finally carry out an interspecific comparison of parturition between the sow and the ewe.

7.2 Pre-parturient phase

7.2.1 Description of behaviour and physiology

The performance of nestbuilding behaviours and the subsequent increase in activity, which was qualitatively described by Jensen (1986) in free-ranging pigs, is apparent from the results of the thesis. Although specific behaviours such as oral manipulation of substrates were not recorded, there is a clear increase in behavioural activity during the 16 hours pre-parturition reaching a peak at around 8 hours pre-parturition. In addition the results from the thesis indicate that this nestbuilding phase is associated with an increase in HPA activity in all gilts. This may be a result of increased behavioural activity (Petrides et al., 1994) however there are additional factors which may also contribute to this increased HPA activity. Uterine contractions will be occurring at this time with increasing frequency and duration (Taverne et al., 1979) and the resulting pain and novelty associated with this could result in increased HPA activity. Therefore the increase in HPA activity seen in all gilts could indicate that factors intrinsic to the pre-parturient phase cause physiological stress and are therefore part of the 'normal' process. Alternatively, it may suggest that neither of the environments used in this study are optimal for a pre-parturient gilt. There may also be some involvement of increased HPA activity with the initiation of parturition. It is clear in the sheep that foetal HPA activity is involved in the initiation of parturition (Liggins et al., 1969), however infusion of piglets with ACTH does not alter the length of gestation and therefore in the pig, the role of foetal HPA activity in the initiation of parturition is unclear (Randall et al., 1990).

7.2.2 Effect of environmental restriction

Irrespective of the potential effect of both crate and pen environments to cause physiological stress, it is clear from the results of the present thesis that the crate system causes a further elevation in HPA activity. This was particularly apparent in the period associated with increased behavioural activity. The crated gilts, however, spent less time standing during the pre-parturient phase but showed elevated levels of plasma cortisol and ACTH in comparison with penned gilts. It is suggested that this may be due to the inability to perform nest-building behaviours as a result of environmental restriction. In addition the crated gilts performed more sitting behaviour during this phase which may reflect motivational conflict (Wood-Gush, 1983). The gilt is unable to perform complex nest-building but at the same time may find lying uncomfortable. The gilt perhaps selects the sitting posture in response to these conflicting motivations.

7.3 Immediate pre-parturient phase

During the two hours before the onset of parturition a decline in activity is seen in gilts housed in both environments. Similarly a decline in plasma cortisol and ACTH is seen, however plasma β-endorphin continues to rise during this period. Evidence in rats suggests that plasma opioids are involved in inducing analgesia at peripheral sites (Joris et al., 1987) and therefore perhaps plasma opioids are involved in the analgesic system which exists in the pig at this time. This opioid-mediated analgesic system may also be involved in the reduction of behavioural activity in the immediate pre-

parturient phase. Results from the present thesis show a rise in pain threshold during the hours immediately prepartum and therefore this may be involved in preventing posture changes due to discomfort. This would allow a period of inactivity before the onset of parturition when frequent uterine contractions and piglet movement within the uterus occur.

7.4 Expulsive Phase

7.4.1 Description of behaviour and physiology

The decreasing behavioural activity seen in the hours immediately prior to the onset of parturition continues during the initial period of the expulsive phase. The general pattern seen during expulsion is a decrease in the time spent standing with an increase in lateral lying. This is accordance with previous studies of pigs in various housing conditions (Jones, 1966b, Randall, 1972, Jensen, 1986). In addition a corresponding change in how the pig interacts with her piglets also occurs during the expulsive phase. During the first hour following the birth of the first piglet the nosing of piglets occurred relatively frequently, however as lateral lying increased during farrowing, results from the present thesis show that the sows became less responsive to piglets. Therefore it appears that overall lateral lying and passivity are major components of maternal behaviour in the pig.

The thesis also provides evidence that this inactivity and passivity may be mediated via endogenous opioids as naloxone, the opioid antagonist, caused an increase in standing and in responsiveness towards piglets. This effect of opioids may be through a number of potential routes (Figure 1). The thesis has described an endogenous analgesic system at parturition in the pig which is partly mediated via endogenous opioids. This system has previously been described in the rat (Gintzler, 1980) and humans (Cogan and Spinnato, 1986, Whipple et al., 1990) however work in the rat has advanced to demonstrate a spinal component (Sander and Gintzler, 1987) and the involvement of Dynorphin A (1-17), an endogenous opioid (Sander et al, 1989). Although the results from the thesis can not conclude the specific opioid responsible for inducing analgesia or the site of action, the thesis provides the first demonstration of an analgesic system in the pig. Therefore the action of opioids on the inactivity of the pig during the expulsive phase may be through their analgesic effects reducing posture changing due to discomfort. There may also be some sedative effect of opioids causing reduced responsiveness not only to piglets but to the environment in general. Opioids have been shown to inhibit oxytocin in the parturient pig (Lawrence et al., 1992, 1996). As oxytocin has been shown to be involved in maternal behaviour of rats (Pedersen and Prange, 1979, Pedersen et al., 1982, Fahrback et al., 1985) the effect of opioids on maternal behaviour in the pig may be through inhibitory effects on oxytocin release.

It is unknown whether pain is involved in the inhibition of oxytocin through resulting increased opioid levels. It has been shown that vagino-cervical stimulation (VCS) causes an increase in oxytocin release in the pig, although opioids do not appear to be

directly involved in these pathways (Gilbert et al., 1997). However VCS may be simply a tactile stimuli and therefore the pathway associated with this may be separate from the ascending pathways associated with painful stimuli such as uterine contraction and expulsion of piglets. It is known that ascending nociceptive pathways terminate in the periaqueductal grey, an area supplied by β-endorphin neurones from the hypothalamus (Basbaum and Fields, 1984). As the inhibition of oxytocin occurs in the hypothalamus (Douglas et al., 1995) this may provide a route for nociceptive stimulation of the uterus and cervix to affect oxytocin release.

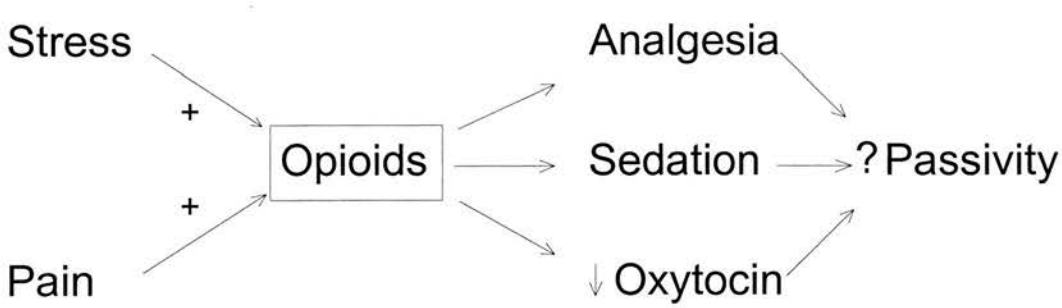


Figure 1. Potential routes for pain and stress, through opioid release, to affect the behaviour of the pig around parturition thereby resulting in passivity. + = potentiating.

7.4.2 Effects of environmental restriction

Results from the thesis indicate no difference in HPA activity between gilts housed in crates and pens during the expulsive phase. This is perhaps unsurprising as the restriction of the crate may be lessened due to the passivity and inactivity of the pig at this time. However, in both environments there is a steady increase in plasma cortisol during the expulsive phase. The function of this rise in plasma cortisol is unknown although there are various factors associated with parturition which may account for this.

The rise in plasma cortisol could reflect increasing motivation for shelter and isolation as the gilt's litter increases which are unattainable in both a crated and penned environment. Therefore the apparent increasing physiological stress may be due to factors which are deficient in both these environments suggesting that neither environment is optimal for a parturient pig.

The present thesis examined the role of piglet expulsion, with the potential for this event to cause physiological stress through resulting pain, stress and novelty. However, results showed that the period following expulsion of a piglet is not associated with an increase in pituitary-adrenal activity.

A further potential stress-inducing factor may be the continuing uterine contractions throughout the expulsive phase. Taverne et al., (1979) showed that these contractions continue, although of shorter duration, at a frequency similar to the pre-parturient period. Therefore contractions plus perhaps movement of piglets in the birth canal could play a role in the increasing plasma cortisol concentrations of the gilt at this time.

The presence of new-born piglets, which are novel to the gilt, may also result in increased plasma cortisol. However, in addition to the presence of piglets there is also a steady increase in suckling during this phase as the number of piglets increases. A study by Lawrence et al., (1994) found a similar increase in plasma cortisol particularly over the first 2 hours following the birth of the first piglet. However, in this study the piglets were removed at birth and returned after placental expulsion. Therefore it appears that the parturient rise in plasma cortisol may be involved in milk production but is not a result of neural input from the suckling stimulus by the piglets.

Cortisol is known to be a catabolic hormone (Moore, 1984) causing an increase in the blood concentration of glucose, amino acids and free fatty acids. Therefore the increased plasma cortisol may be required to allow these metabolites to be available for further milk production. If this is the case then the opioid mediation of the HPA axis, previously found in non-pregnant gilts (Rushen and Ladewig, 1991, Rushen et al., 1993), would be disadvantageous as opioid levels are high at this time and would

therefore inhibit HPA activity. Results from the thesis suggest that the opioid-mediation of the HPA axis is removed in parturient pigs and therefore perhaps reinforces the suggestion that the increase in plasma cortisol may be part of 'normal' parturition. Oestrogen, which increases at farrowing (Robertson and King, 1974, Robertson et al., 1985, McLean et al., in press) has been shown to be involved in resetting the negative feedback of the HPA axis (Nolten et al., 1980), and therefore may be involved in potentiating the release of cortisol to facilitate subsequent milk production.

Due to this, as yet unexplained, rise in plasma cortisol concentration during the expulsive phase it is necessary to investigate these potential factors to ensure that this is part of a 'normal' parturition and not a result of some adverse effect of the environments in which parturient pigs are housed.

7.5 Evolutionary aspects of maternal behaviour in the pig

7.5.1 Maternal care

The description of maternal behaviour provided in the thesis suggests that behaviours indicative of good maternal care in other species are not performed by the pig. The sows observed in the thesis were generally inactive and unresponsive to piglets during the expulsive phase. When we consider this passivity and inactivity in relation to the behaviour of the pig in free-ranging conditions, as described by Jensen (1986), we can perhaps begin to understand why this strategy has been adopted by the pig. The pig is a nesting animal, and has numerous precocial young which are small in relation to the dam and therefore the main risks to her piglets are starvation, hypothermia, crushing and predation.

It is established that pigs do not lick their young (Jones, 1966b, Randall, 1972) thereby do not remove the amniotic fluids. It is crucial therefore that the piglets have access to the udder for warmth and nutrition, and it was suggested by Fraser (1984) that remaining in lateral recumbency was to allow full udder exposure for this purpose. In addition to allowing access to the udder, I suggest that the amount of time spent in lateral recumbency may have other benefits, namely: a reduction in the number of posture changes prevent crushing of piglets, less disturbance of the nest structure and the reduced risk of attracting predators to the nest.

The results of the thesis indicate endogenous opioids as neural substrates for this inactivity and passivity. However, I have suggested that this analgesic system, due to the increase in opioid levels, is involved in inducing this inactivity and passivity; a strategy adopted by the pig to maximise the survival of her piglets. Therefore if we now regard passivity and inactivity as components of good maternal care we must then consider the adverse effects on mother-young bonding. It appears that there is some trade-off between remaining inconspicuous and inactive during expulsion with a subsequent reduction in mother-young bonding. However if we consider that the sow and her litter remain isolated in the nest for around 10 days (Jensen, 1986) following farrowing, perhaps bonding may not be of the greatest importance during the expulsive phase.

In my opinion the inactivity and passivity seen in the pig during farrowing are components of good maternal care and therefore the pig differs from other species in this respect. This strategy may have been adopted with the primary adaptive value being to maximise the survival of piglets at this time with a reduction in the bonding of the sow with her young

The identification of behaviour representing good maternal care in the pig is of extreme importance with regard to the selection of pigs for increased rearing ability. Perhaps in the case of the pig it is more suitable to select for decreased responsiveness or to select against maternal aggression.

7.5.2. Comparison with the behaviour and physiology of sheep

Sheep perform behaviours towards their young in the immediate post-natal period which are more widely regarded as indicative of good maternal care; licking, sniffing, low-pitch bleating (Dwyer et al., in press). However when we consider the behaviour of the ewe around parturition there are some vast differences between the sheep and the pig. Similarly to the pig, the ewe will leave the social group to give birth in isolation (Lynch et al., 1992) however the ewe does not construct a nest in which to give birth as the pig does (Jensen, 1986). The pig will select a nesting site normally in a sheltered area (Jensen, 1986) however the ewe, if the weather conditions are suitable, may select a more elevated area near fencelines (Lynch et al., 1992). The expulsive phase of parturition takes considerably less time in the ewe: around 1-2 hours (Lynch et al., 1992), compared with the pig which can take up to 16 hours (Signoret, 1975). The lamb is large in terms of proportion of dam size and the litter size of sheep is normally one or two, depending on the breed. In addition, ewes of domestic breeds normally rejoin the flock within 12 hours of parturition (Lynch et al., 1992) once the ewe and lamb have bonded, mediated by the licking and low pitched bleating of the ewe.

The underlying physiology of the two species also differs; as mentioned opioids inhibit oxytocin in the pig, however the opposite is the case for the sheep. Opioids have been shown to enhance maternal behaviours in the ewe (Keverne and Kendrick,

1991). Therefore opioids may potentiate the large release of oxytocin seen at the onset of parturition in the sheep (Keverne and Kendrick, 1992) allowing her to give birth to her small litter quickly. In the pig the expulsion of numerous piglets over several hours requires that oxytocin is released over a longer period of time following the onset of parturition (Lawrence et al., 1996). A study by Gilbert et al., (1994) provides evidence of pulsatility of oxytocin release into the periphery. Perhaps the pulse rate is a result of repeated opioid inhibition followed by desensitisation of the oxytocin neurones as naloxone has been shown to increase the pulse rate of oxytocin release (Gilbert et al., 1997).

Vocalisations are an important part of bonding of the ewe and her lamb (Dwyer et al., in press) and therefore are necessary before the movement away from the parturition site. Opioids have been shown to inhibit distress vocalisations in the guinea pig (Herman and Panksepp, 1981), therefore any opioid-mediated inhibition of the ewe's vocalisations may attenuate the bonding between the ewe and her lamb.

Therefore there are a number of differences in the strategies used by sheep and pigs to increase the survival of their offspring. Future research should explore how the underlying physiology associated with parturition relates to these divergent behavioural responses, and in particular the differing role that opioids may play with regard to the maternal behaviour of these two species.

Conclusions of the thesis

Late pregnancy and parturition in the pig is associated with an endogenous analgesic system which is, at least in part, mediated via endogenous opioids.

Passivity and inactivity are major components of maternal behaviour in the pig and are suggested to be indicative of good maternal care. The present thesis provides evidence of an opioid mediation of maternal behaviour which may arise through several potential routes, namely: the analgesic system, sedation, inhibition of oxytocin.

A rise in plasma cortisol, ACTH and β-endorphin concentrations were found in pre-parturient gilts housed in both straw bedded pens and conventional farrowing crates. However, crates caused further stimulation of the HPA axis perhaps reflecting thwarting of nestbuilding behaviour in this restrictive environment.

The farrowing crate did not cause further HPA activity during the expulsive phase which may reflect the inactivity of the pig at this time. A rise in plasma cortisol was found as the expulsive phase progressed irrespective of environment however the thesis found that the expulsion of a piglet does not appear to play a major role in this.

Overall the thesis has advanced our understanding of parturition in the pig by relating the physiology and behaviour of the pig at this time. The thesis has also highlighted welfare implications regarding the use of farrowing crates, and provides information which may be used when considering changes to housing for parturient pigs.

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List of publications

1. S Jarvis , K McLean, J Chirnside, L Deans, S Calvert, V Molony, A B Lawrence (1995). Opioid-mediated changes in pain threshold throughout pregnancy and parturition in the sow. Journal of Reproduction and Fertility, Abstract Series no. 16, p13.

2. S Jarvis, A B Lawrence, K McLean, L Deans, J Chirnside, S Calvert (1996). The effect of environment on behavioural activity, ACTH, β-endorphin and cortisol in pre-farrowing gilts. Animal Science **62**, p629-630.

3. S Jarvis, K McLean, S Calvert, J Chirnside, L Deans, A B Lawrence, V Molony (1996). Changes in pain threshold during pregnancy and parturition in the sow and the involvement of opioids. Animal Science **62**, p628-629.

4. S Jarvis, KA McLean, J Chirnside, LA Deans, SK Calvert, V Molony, AB Lawrence (1997). Opioid-mediated changes in nociceptive threshold during pregnancy and parturition in the sow. Pain, 72, 153-159.

5. S Jarvis, AB Lawrence, KA McLean, LA Deans, J Chirnside, SK Calvert. The effect of environment on behavioural activity, ACTH, β-endorphin and cortisol in pre-farrowing gilts. Animal Science, 65, 465-472.

1. Opioid-mediated changes in pain threshold throughout pregnancy and parturition in the sow. S Jarvis, K McLean, J Chirnside, L Deans, S Calvert, V Molony, A B Lawrence (1995). Journal of Reproduction and Fertility, Abstract Series no. 16, p13.

This study aimed to examine changes in pain threshold in pigs during pregnancy and parturition, and to determine whether any changes seen were opioid-mediated. Sixteen Large White x Landrace multiparous sows were tested in straw bedded pens (2.5m x 2.5m) during weeks 4, 8 and 12 of pregnancy and over the farrowing period. Testing involved thermal stimulation of 8 areas on the rear-quarters of the sows with a CO_2 infra-red laser until a physical response was seen (tail flick, leg move, muscle twitch) or for a maximum of 16 seconds. Over the farrowing period testing was more frequent, and at 4 hours after the birth of the first piglet, half the sows received an injection (i.m.) of naloxone (N) (1mg kg^{-1} body weight) with the remainder receiving a control dose of saline (S). Responses were recorded 15 and 30 minutes post-injection. There was no significant difference between response times throughout weeks 4, 8 and 12 (mean response time (mrt) (seconds) ± s.e.m.: 8.26 ± 0.18, 10.07 ± 0.23, 9.48 ± 0.21 for weeks 4, 8 and 12 respectively, $F_{2,26}=0.97$, $p<0.152$), however a significant rise was seen from week 12 (baseline) to 5 days before parturition (mrt: 12.07 ± 0.31 for 5 days pre-farrowing, $W=102$, $p<0.002$). Response times continued to rise above baseline until the birth of the first piglet by which time the majority of sows had stopped responding within 16 seconds (mrt: 15.77 ± 0.12 at the birth of the first piglet, $W=136$, $p<0.001$). By 48 hours after the birth of the first piglet response times fell to levels seen 5 days before parturition. After administration of naloxone response times fell compared to control animals at 15 mins (mrt: 15.58 ± 0.21 and 13.52 ± 0.48 for S and N sows, $W=4660.5$, $p<0.001$) and 30 mins (mrt: 15.87 ± 0.09 and 14.79 ± 0.34 for S and N sows, $W=4028.5$, $p<0.01$) post-injection. These results suggest that late pregnancy is associated with an increase in opioid tone which alters pain threshold in the sow, perhaps as an endogenous defence against labour pain. Endogenous opioids are known to inhibit oxytocin release at parturition, thus the effects of pain-induced opioid activity on oxytocin release should be further investigated.

2. The effect of environment on behavioural activity, ACTH, β-endorphin and cortisol in pre-farrowing gilts. S Jarvis, A B Lawrence, K McLean, L Deans, J Chirnside, S Calvert (1996). Animal Science 62, p629-630.

This study examined the temporal relationships between behavioural activity and hormones commonly associated with stress and pain in gilts farrowing in two environments. Thirty one Large White X Landrace gilts with indwelling jugular catheters were blood sampled (0800 and 1600 h) from 10 days before their expected parturition date (EPD). Five days before EPD they were moved to either a farrowing crate (C) with no bedding, or a pen (P) (2.5m x 3.0m) with straw provided (blood sampled at 0800, 1200 and 1600 h). Around 12 hours before the onset of farrowing blood samples were taken remotely at ½ hour intervals using a catheter extension. The posture of the gilts was recorded using 5 minute scan samples over the 24 hours pre-farrowing. The proportion of scans standing (an index of activity) was strongly affected by time ($p<0.001$) with peak levels at approximately 7-8 hours pre-farrowing in both treatments, and by treatment (0.25 v. 0.33 for C and P gilts respectively; s.e.d. 0.03, $p<0.05$). Plasma cortisol concentrations also rose before farrowing ($p<0.001$) reaching a peak at 12-6 hours pre-farrowing. Crated gilts had higher cortisol concentrations than penned gilts (overall mean: 41.5 v. 30.7 ng ml^{-1} for C and P gilts respectively; s.e.d. 3.8, $p<0.05$) at 24-12 ($p<0.05$), 12-6 ($p<0.01$) and 6-2 ($p<0.05$) hours pre-farrowing. Plasma ACTH concentration showed a similar pattern to cortisol over the pre-parturient period, peaking at 12 hours pre-farrowing in both treatments (time: $p<0.001$); crated gilts had significantly higher concentrations of ACTH at 6 hours pre-farrowing only ($p<0.05$). Plasma β-endorphin concentrations also showed a gradual rise ($p<0.001$) towards parturition; however no treatment differences were seen. These results suggest that the pituitary-adrenal (PA) axis is stimulated during pre-farrowing activity irrespective of farrowing environment. Crates, without bedding, further stimulate the PA axis over the pre-farrowing period perhaps by preventing nest-building. The rise in ß-endorphin may be involved in an endogenous defence against parturition pain.

3. Changes in pain threshold during pregnancy and parturition in the sow and the involvement of opioids. S Jarvis, K McLean, S Calvert, J Chirnside, L Deans, A B Lawrence, V Molony (1996). Animal Science 62, p628-629.

This study aimed to demonstrate pregnancy-induced analgesia in the sow, and to examine the role of endogenous opioids which are known to be released in response to pain. Sixteen Large White x Landrace multiparous sows were tested in straw bedded pens (2.5m x 2.5m) during weeks 4, 8 and 12 of pregnancy and over the farrowing period. Testing involved thermal stimulation of 8 areas on the rear-quarters of the sows with a CO_2 infra-red laser until a physical response was seen (tail flick, leg move, muscle twitch) or for a maximum of 16 seconds. Over the farrowing period testing was more frequent, and at 3¾ hours after the birth of the first piglet, half the sows received an injection (i.m.) of naloxone (N), an opioid antagonist, (1mg kg^{-1} body weight) with the remainder receiving a control dose of saline (S). Responses were recorded 15 and 30 minutes post-injection. There was no significant difference between response times over weeks 4, 8 and 12 ($F_{2,26}=0.97$, $p<0.152$), however a significant rise was seen from week 12 (baseline) to 5 days before parturition ($W=102$, $p<0.002$). Response times continued to rise above baseline until the birth of the first piglet by which time the majority of sows had stopped responding within 16 seconds ($W=136$, $p<0.001$). Response times fell during days 1, 2 and 7 post-partum. After administration of naloxone response times fell compared to control animals at 15 mins (mrt: 15.58 ± 0.21 and 13.52 ± 0.48 for S and N sows, $W=4660.5$, $p<0.001$) and 30 mins (mrt: 15.87 ± 0.09 and 14.79 ± 0.34 for S and N sows, $W=4028.5$, $p<0.01$) post-injection. These results suggest that pain threshold increases during late pregnancy in the sow, perhaps as an endogenous defence against labour pain, and that during parturition this change in pain threshold is, at least in part, opioid-mediated. Oxytocin is known to be inhibited by endogenous opioids at parturition, thus future research should consider the potential role of birth pain as a negative feedback to oxytocin release.

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PAIN

Opioid-mediated changes in nociceptive threshold during pregnancy and parturition in the sow

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Abstract

This study aimed to investigate if pregnancy-induced hypoalgesia occurs in the sow, and to examine the role of endogenous opioids which are known to be released in response to nociception. Sixteen Large White × Landrace multiparous sows were tested in straw bedded pens (2.5 × 2.5 m) during weeks 4, 8 and 12 of pregnancy and over the farrowing period. Testing involved thermal stimulation of eight areas on the rear-quarters of the sows with a CO_2 infra-red laser until a physical response was seen (tail flick, leg move or muscle twitch) or for a maximum of 16 s. Over the farrowing period testing was more frequent, and at 3.75 h after the birth of the first piglet, half the sows received an injection (i.m.) of an opioid antagonist naloxone (N) (1 mg kg^{-1} body weight) with the remainder receiving a control dose of saline (S). Responses were recorded 15 and 30 min post-injection. There was no significant difference between response times over weeks 4, 8 and 12 of pregnancy ($P = 0.152$), however a significant rise was seen from week 12 to 5 days before parturition ($P = 0.002$). Response times continued to rise until the birth of the first piglet by which time the majority of sows had stopped responding within 16 s ($P < 0.001$). Response times fell over days 1, 2 and 7 post-partum. After administration of naloxone response times fell compared to control animals at 15 min ($P < 0.001$) and 30 min ($P < 0.01$) post-injection. These results suggest that nociceptive threshold increases during late pregnancy in the sow, perhaps as an endogenous defence against labour pain, and that during parturition this change in nociceptive threshold is, at least in part, opioid-mediated. Oxytocin is known to be inhibited by endogenous opioids at parturition, thus future research should consider the potential role of increased nociception at birth as a negative feedback to oxytocin release.

Keywords: Sow; Pregnancy; Hypoalgesia; Parturition; Opioids; Pain

1. Introduction

The human birth process is associated with increased nociception and has consequently received a great deal of scientific interest. However there has been very little interest in the pain experienced by farm animals during parturition. With the genetic selection for faster growth leading to increased size of offspring, there are potential welfare implications for the mother, both in terms of the progress of parturition and the onset of maternal behaviour.

An increase in nociceptive threshold has been shown in both women (Cogan and Spinnato, 1986; Whipple et al., 1990) and in the rat (Gintzler, 1980) perhaps as an endogenous defence against the pain of parturition. Whipple et al. (1990) showed that tactile threshold did not change over the parturient period indicating that the changes seen in nociceptive threshold were not due merely to distraction caused by the labour process itself. Endogenous opioids are known to be released in response to nociception, and have potent analgesic properties (Dalayeun et al., 1993), and Gintzler (1980) suggested that the pregnancy-induced hypoalgesia observed in the rat was mediated via endogenous opioids as the rise in nociceptive threshold did not occur in rats administered (s.c.) with naltrexone, an opioid antagonist. A further study in rats suggested that a spinal opioid mechanism was involved as a significant decrease in nociceptive threshold was seen when naltrexone was administered intrathecally (i.t.) (Sander and Gintzler, 1987).

Neural supply to the uterus and cervix is mainly via the

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hypogastric and pelvic nerves which enter the spinal cord through dorsal roots predominantly in the lumbar region (Steinman et al., 1992; Berkley et al., 1993), and therefore this area may be particularly involved in the hypoalgesia associated with late pregnancy. Transection of the hypogastric nerve significantly reduced the increase in nociceptive threshold in rats over the pre-parturient period compared to sham operated animals (Gintzler et al., 1983). Pelvic neurectomy in rats also causes attenuation of the rise in nociceptive threshold associated with uterocervical mechanostimulation (Gintzler and Komisaruk, 1991). Elevated levels of dynorphin A (1–17), an opioid which preferentially binds to κ-receptors, were found in the lumbar region on both day 22 of pregnancy and during parturition in the rat (Medina et al., 1993a), with levels unchanged in the cervical and thoracic areas of the spinal cord. In addition intrathecal administration of a κ specific antagonist, nor-binaltorphimine, has been shown to reduce nociceptive threshold in rats on Day 20 of pregnancy (Sander et al., 1988). Thus it is likely that uterine distension and cervical stretching, which occur during the later stages of pregnancy and parturition, are involved in activation of pregnancy-induced hypoalgesia by stimulation of afferents in the hypogastric and pelvic nerves. It also appears that this pregnancy-induced hypoalgesia is mediated via a spinal κ-opioid mechanism. However further work in the rat has suggested that simulation of pregnancy by administration of 17β-estradiol and progesterone modulates an opioid analgesic system (Dawson-Basoa and Gintzler, 1993), and also increases spinal cord dynorphin A (1–17) in the lumbar region of the spinal cord (Medina et al., 1993b). Therefore, both hormonal and neuronal factors may be involved in the activation of pregnancy-induced hypoalgesia.

It has been shown initially in the rat (Leng et al., 1988) and then in the pig (Lawrence et al., 1992) that environmental stress inhibits oxytocin and slows delivery and that both of these effects are naloxone-reversible. Thus opioids released in response to increased nociception at parturition, perhaps due to an increase in the size of offspring, may also act to inhibit oxytocin and may compromise the welfare of the sow by prolonging parturition and interfering with maternal behaviour. Therefore this study aimed to initially determine whether changes in nociceptive threshold occurred over pregnancy and parturition in the sow, indicative of pregnancy-induced hypoalgesia. Secondly, we wished to demonstrate whether any changes were mediated via an opioid mechanism, with a view to further research considering the potential for increased nociception at parturition to modify oxytocin release.

2. Animals, materials and methods

2.1. Animals and housing

The experimental protocol was reviewed and approved by the Animal Experiments Committee of the Scottish Agricultural College, Edinburgh, UK, and the procedures used were in accordance with the Animals (Scientific Procedures) Act (1986).

The subjects of this study were 16 Large White × Landrace sows (Easter Howgate Pig Unit, Milton Bridge, Penicuik, Midlothian, UK; mean parity ± SE, 3.7 ± 0.04) studied in two replicates of seven and nine sows, respectively. The sows were initially group housed in straw-bedded pens (2.6 × 4.1 m) and fed 2.5 kg day^{-1} of a commercial diet providing 13 MJ DE kg^{-1} at 0800 h. A boar was introduced daily and was used to serve the sows on 2 consecutive days. The expected parturition day (EPD) was calculated as 114 days (16.3 weeks) after the first service. Once pregnancy was confirmed at around 32 days after service, the sows were housed in groups of three or four in a semi-open building in a pen consisting of a concrete yard (6.0 × 4.0 m) with a straw bedded kennel area at the back (6.0 × 1.5 m). The sows were floor fed 2.5 kg day^{-1} of the same commercial feed at 0800 h. Artificial lighting was provided between 0730 and 1700 h.

2.2. Experimental housing

For testing, sows were moved with their group to a temperature controlled room (18°C) for approximately 1 week. This occurred at weeks 4, 8 and 12 of pregnancy, with artificial lighting provided as before. Each sow was moved to an individual straw bedded pen (2.5 × 2.5 m) and trough fed the same feed at the previous amount and time. These pens were also used for the sows to farrow (give birth), at which time a creep (heated area for piglets) was added. Over the farrowing period the feed was changed to a commercial diet providing 13.75 MJ DE kg^{-1} and containing 18% protein. This lactation diet was offered in two meals at 0800 and 1600 h. The level of feed increased from 2.5 kg day^{-1} depending on time since farrowing.

2.3. Measurement of nociceptive threshold

The noxious stimulus applied in this study to measure nociceptive threshold was a thermal stimulus produced by a CO_2 infra-red laser (MPB Technologies, Dorval, Quebec, Canada). The power setting decided upon (1.5 W) was that which produced a mean skin temperature of 52.3°C ± 0.76 (SEM). In a pilot study using non-test sows this setting consistently gave rise to behavioural responses at around 8–10 s, however after 15 s skin damage occurred. Therefore 15 s was used as the end point. The specific areas on the rear end of the sows which were to be stimulated with the laser were also decided upon within this pilot study (Fig. 1). The areas were shaved and marked clearly with a permanent pen, and this pattern of areas was used for all sows throughout the experiment. Within one test the eight areas were stimulated at random. The heat producing beam worked in

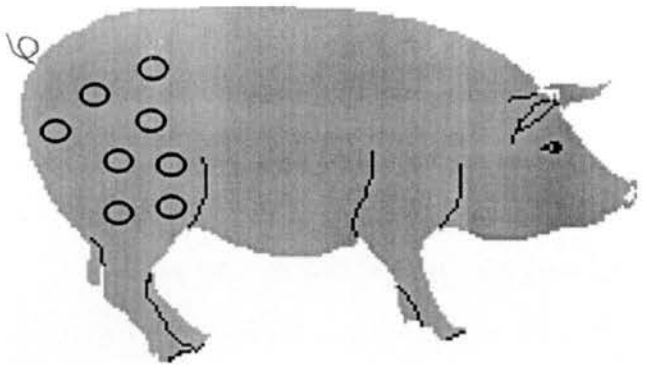

Fig. 1. Pattern of areas used on all sows (n = 16) over the entire study.

conjunction with a timer, such that when the observer started the timer the heat producing beam came on automatically. When a physical response was observed (tail flick, movement of the back leg, muscle twitch), the timer was stopped and the time and nature of the response was recorded. If no response was observed within the 15 s, the laser automatically stopped, and a value of 16 s was allocated for statistical purposes.

The surface temperature of each area was recorded using a remote infra-red thermometer (Cyclops Compac 3, Minolta/Land, Sheffield, UK) before stimulation with the laser to determine the effect of initial skin temperature on the response time of the pig. All reflective objects were removed from the room and all safety procedures were employed in accordance with the University of Edinburgh Radiation Protection Committee (The University of Edinburgh, 1993).

2.4. Heart rate measurements

A further pilot study on non-test sows was carried out to measure heart rate over the 15 s stimulation to determine any stress-inducing effects of the test. This involved using a heart rate monitor (Polar Electro PE3000, Kempele, Finland) which was attached to the pigs during the 15 s laser stimulation. The heart rate was measured just prior to each stimulation, and then 5, 10 and 15 s during the stimulation. The data was analysed using a repeated measures analysis of variance.

2.5. Pregnancy up to week 12

After sows had been moved to the test pens at weeks 4, 8 and 12 of pregnancy, they were allowed a 2 day habituation period. Following this period the laser tests were carried out on 3 alternate days in week 4, and on 2 alternate days in weeks 8 and 12. On each test day, one side of the pig was tested in the morning and the other side in the afternoon. The sides were tested in the opposite order on the following test day. The sows were only tested when they were in a lying position and were awake. All laser sessions were recorded on video (Panasonic 24 h time lapse video recorder, AG-6024).

2.6. Parturition

The sows were moved into the test pens 7 days before the EPD. Tests were carried out, again in the morning and afternoon, on day −5 (5 days before EPD) and continued on consecutive days until day −3. On day −2 the morning and afternoon tests were carried out, and the sows were also tested at 2400 h. They were then tested every 8 h from this point until nest-building began, at which point tests were carried out every 4 h until the delivery of the first piglet. The indicators used to determine the onset of nest-building were oral manipulation of straw, bars and the trough. If nest-building began before midnight on day −2, 4 h tests were started from the onset of nest-building. A laser test was carried out following the birth of the first piglet (mean ± SE, 13.43 ± 5.1 min post-piglet 1). Testing was also carried out 2 and 6 h after the first piglet was born. Alternating sides for testing was difficult at this time due to the sows tending to lie on the same side during parturition, therefore tests were carried out on the exposed side. Morning and afternoon tests were resumed on days 1, 2 and 7 post-partum. All laser tests were recorded on video, and piglet weight, sex and time of birth were recorded. Responses to stimulation were measured on six of the 16 sows post-weaning, which was 1 day after the piglets had been removed following a 28 day lactation period.

2.7. Effect of naloxone

To test the involvement of opioids, naloxone, an opioid antagonist, was administered (1mg kg^{-1} body weight) intramuscularly in the neck to half of the sows at 3.75 h after the birth of the first piglet, and a laser test carried out 15 and 30 min post-injection (4 and 4.25 h after the first piglet). The other half of the sows received saline as a control and were tested in the same way post-injection.

2.8. Observers

Five observers were required to conduct this study to allow night work to be carried out. The experiment was, as mentioned earlier, carried out in two replicates, therefore reducing the possibility that changes in response times were due to the improved definition of responses by observers. Also within each replicate the sows EPDs were staggered over time so that pregnancy and parturition measurements were being carried out simultaneously.

2.9. Statistical analysis

2.9.1. Pregnancy

The data over pregnancy were normally distributed and were analysed by a repeated measures analysis of variance

(Genstat, Version 5, Lawes Agricultural Trust, Rothamsted Experimental Station) using a blocked structure for replicate, pig, week and time of day. Factors used were week (4, 8, 12), time of day (am, pm), area (eight levels), replicate (two levels) and parity (six levels). The effect of the observer could not be added into the blocking structure as it was unbalanced, therefore a regression analysis (Minitab, version 7.1) was performed between observer and response time to determine any effect. A second regression analysis was performed between initial skin temperature and response time.

2.9.2. Parturition

The data obtained over the parturition period were not normally distributed due to a large proportion of the observations being at the 16 s cut-off point, and therefore non-parametric statistics were applied. Initial skin temperature and observer were not accounted for over this period as analysis of the pregnancy data showed that neither of these variables affected response time. The data were divided into responses at specific times or responses pooled over time periods (Table 1). In order to analyse temporal changes in nociceptive threshold the difference between two successive times was calculated and a Wilcoxon Rank Sign Test (Minitab, version 7.1) used to determine whether the change was significantly less or greater than zero.

2.9.3. Pregnancy and parturition

A Wilcoxon Rank Sign test (Minitab, version 7.1) was used to determine changes in response time between week 12 of pregnancy and 5 days pre-parturition. The post-weaning values recorded for six sows were compared, using a paired *t*-test, with the mean values over pregnancy for the six sows.

Table 1

Description of pooled time periods and specific time points around parturition used in the statistical analysis

Time Pooled Periods (frequency of tests)	Specific times	Description
Day −5 (twice daily)		−5 days
Day −4 (twice daily)		−4 days
Day −3 (twice daily)		−3 days
−48 to −24 h (8 h)		−48 h
−24 to −12 h (8 h)		−24 h
−12 h to the birth of piglet 1 (4 h)		−12 h
	Birth of piglet 1 (P)	0
	P + 2 h	+2 h
	P + 6 h	+6 h
	P + 12 h	+12 h
Day +1 (twice daily)		+1 day
Day +2 (twice daily)		+2 days
Day +7 (twice daily)		+7 days
Post-weaning (twice daily)		pw

2.9.4. Effect of naloxone

A Mann–Whitney test (Minitab, version 7.1) was used to compare response times of sows receiving naloxone and those receiving saline at both 15 and 30 min post-injection.

3. Results

3.1. Length of parturition, litter size and weight

The mean duration of parturition was 3.99 ± 0.67 (SE) h, with the mean inter-piglet birth interval being 23.77 ± 3.70 (SE) min. The effect of naloxone on these variables could not be determined as most sows had had their piglets by the time of the injection. The mean litter size was 11.13 ± 0.74 (SE) and the mean weight of the piglets was 1.59 ± 0.08 (SE) kg.

3.2. Heart rate measurements

There was no effect of laser stimulation on the heart rate of the non-test sows in the pilot study. The mean heart rates (±SE) during the laser stimulation were 85.04 (±1.42), 85.05 (±1.38), 85.44 (±1.37) and 85.37 (±1.43) for 0, 5, 10 and 15 s respectively. This suggests that a rise in response time is not due to any stress-inducing effects of the test.

3.3. Pregnancy

Response times did not change significantly during early and mid pregnancy (mean response time (s) ± SE, 8.26 ± 0.56 for week 4, 10.04 ± 0.63 for week 8 and 9.45 ± 0.58 for week 12).

A regression of the initial skin temperature of each area on response time showed that temperature accounted for less than 1% of the variation in response time ($R^2 = 0.6\%$, $t = 0.3$). Observer also explained less than 1% of the variation in response time ($R^2 = 0.8\%$, $t = -3.72$).

3.4. Parturition

As can be seen in Fig. 2, there was an increase in response time between week 12 of pregnancy and −5 days ($W = 102$, $P < 0.01$). A significant rise in response time can be seen between −3 days and −48 h ($W = 117$, $P < 0.05$), and also between −24 h and −12 h ($W = 101$, $P < 0.05$) (Fig. 2). At the birth of the first piglet, a highly significant increase in response time had occurred relative to −12 h ($W = 136$, $P < 0.001$). The mean response time had reached the maximum value of 16 s (no response to the laser) for most sows and remained at this level until +2 h. A slight decrease in response time was found between +2 h and +6 h ($W = 3.0$, $P < 0.05$). A gradual decline in response time was seen over +1 day, +2 days and +7 days. By the post-weaning period, the response times of the six sows tested did not differ significantly from +7 days. Also the response times

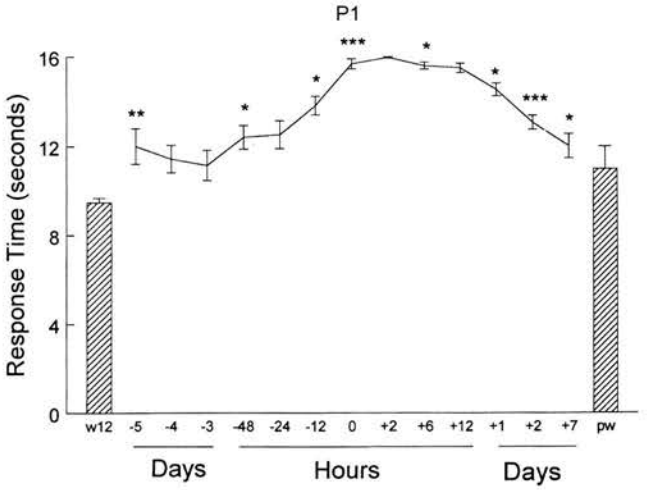

Fig. 2. Mean response times (± SE) over the farrowing period (n = 16). A mean for week 12 (w12) is depicted to indicate levels during pregnancy (n = 16). A mean for the post-weaning (pw) measurements (n = 6) is also given. Significance levels at a time period indicate whether a rise or fall in response time has occurred from the previous time period. P1, piglet 1. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

of the six sows at post-weaning were significantly higher ($T = 2.92$, $P < 0.05$) than their response times during pregnancy.

3.5. Effect of naloxone

At 15 min after the injection, most sows receiving saline did not respond within the 15 s stimulation (16 s allocated), whereas the response times of sows receiving naloxone had fallen significantly ($W = 4660.5$, $P < 0.001$) (Fig. 3). The sows receiving naloxone also had significantly lower response times ($W = 4028.5$, $P < 0.01$) at 30 min post-injection. There was no difference between the response times of the allocated groups at +2 h (1.75 h prior to the injection). Again at +6 h (2.25 h post-injection) there was no difference between the response times of the allocated groups suggesting no persisting effects of naloxone.

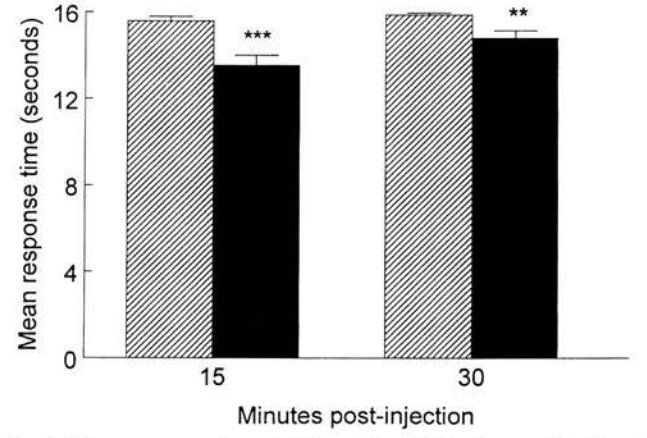

Fig. 3. Mean response times (+SE) at 15 and 30 min post-injection of either saline (stripped bar (n = 8) or naloxone (black bar (n = 8). $^{**}P < 0.01$, $^{***}P < 0.001$.

4. Discussion

The results of this study suggest that hypoalgesia occurs during late pregnancy and parturition in the sow. The attenuation of the rise in nociceptive threshold seen by naloxone suggests that an opioid mechanism is partly involved at least during parturition. This provides further evidence for pregnancy-induced hypoalgesia which has previously only been demonstrated in the rat (Gintzler, 1980) and women (Cogan and Spinnato, 1986; Whipple et al., 1990). Response times did not increase as a result of the repeated use of the noxious stimulus, as firstly the response times did not increase over the first 12 weeks of pregnancy, and secondly a decline in response time was observed in the post-partum period.

The pharmacokinetics of naloxone have not been studied in the pig; in sheep however it has been shown to have a half-life of around 40 min (Alavi et al., 1994). This short half-life may account for the reduction in significance level between 15 and 30 min post-injection. Although the fall in response time seen in the sows receiving naloxone was significant, the reduction appears to be relatively small. This may be partly a result of the cut-off point being set at 15 s, as the actual response times of the saline animals may have been considerably longer. Also naloxone did not fully reverse the rise in nociceptive threshold suggesting that opioids are only partly responsible for hypoalgesia during parturition or more simply that the dose and route of administration used in this study was insufficient.

As naloxone was administered intramuscularly in this study, the site of action can not be determined. This is also the case for the work carried out in rats by Gintzler (1980), as naltrexone was administered subcutaneously. However it has been shown that intrathecal administration of naltrexone in pregnant rats causes a decrease in nociceptive threshold suggesting a spinal opioid pathway is involved in pregnancy-induced hypoalgesia (Sander and Gintzler, 1987).

Naloxone exhibits some preference for μ-receptors (Herz and Almeida, 1989), however has been shown to bind to κ-receptors (Herkenham et al., 1986) and δ-receptors (Sharif and Hughes, 1989). Therefore the specific opioid ligand responsible for this pregnancy-induced hypoalgesia in the pig cannot be concluded. Work in rats has shown that norbinaltorphimine (i.t.), a κ-selective antagonist reduced nociceptive threshold of rats to an electric foot-shock during day 20 of pregnancy (Sander et al., 1988), suggesting the involvement of a κ-opioid system. Dynorphin A (1–17), an opioid which preferentially binds to κ-receptors, has been shown to increase in the lumbar region of the spinal cord during day 22 and parturition in the rat (Medina et al., 1993a). The main afferent nerves of the uterus and cervix, the hypogastric and pelvic nerves, enter the spinal cord through various dorsal roots, but predominantly in the lumbar region where, as mentioned, dynorphin A (1–17) levels increase around parturition. Work involving transection of

the hypogastric nerve during pregnancy and parturition (Gintzler et al., 1983), and pelvic neurectomy prior to uterocervical mechanostimulation (Gintzler and Komisaruk, 1991) reduced hypoalgesia. The hypogastric nerve terminal fields are in lamina I and V of the dorsal horn, which is where dynorphin A (1–17) is predominantly found (Dubner and Hargreaves, 1989), and lamina I is very rich in opiate receptors (Sander and Gintzler, 1987). It is suggested therefore that uterine distension and cervical stretching, which are essential parts of the parturition process, stimulate afferents running in the hypogastric and pelvic nerves and activate a spinal, probably κ, opioid system.

Results obtained in this study suggest that response times in the sow have increased significantly by at least 5 days pre-parturition, i.e. before the onset of uterine contractions and cervical stretching. Other studies indicate that the activation of pregnancy-induced hypoalgesia may also be under endocrinological control. Simulation of pregnancy using 17-β oestradiol and progesterone results in hypoalgesia in rats (Dawson-Basoa and Gintzler, 1993), with nociceptive thresholds showing a similar pattern to that seen in pregnant rats (Gintzler, 1980). Both studies showed that the increase in nociceptive threshold was naltrexone reversible suggesting involvement of an opioid mechanism. Dynorphin A (1–17) levels increased in the lumbar region of the spinal cord when pregnancy was simulated with 17-β oestradiol and progesterone (Medina et al., 1993b), suggesting that this opioid appears to be involved in pregnancy-induced hypoalgesia. A more recent study has suggested that this simulation of pregnancy does activate spinal κ-opiate receptor analgesic mechanisms (Dawson-Basoa and Gintzler, 1996). Therefore pregnancy induced changes in sex-steroid concentrations may activate pregnancy-induced hypoalgesia, whilst uterine distension and cervical stretching accentuate the hypoalgesia around parturition. A further accentuation of hypoalgesia has also been shown after ingestion of placenta (Kristal et al., 1985) and amniotic fluids (Kristal et al., 1990) in rats. However the placenta was removed in the present study and cannot account for changes in nociceptive threshold.

The response times of the sows at 1 day after weaning was significantly higher than those recorded during pregnancy, however this may have been due to stress associated with the separation from their piglets. Previous work in rats has shown that nociceptive thresholds are low during lactation but increase following weaning suggesting stress-induced analgesia at this time (Cruz et al., 1996).

Pregnancy-induced hypoalgesia results from, at least in part, an increased level of opioids in response to both hormonal and neuronal factors which occur at this time. The consequence of these opioids may not be solely the modification of the perception of pain. Evidence suggests that opioids inhibit oxytocin release from the neurohypophysis in the rat (Bicknell and Leng, 1982), and both μ and κ opioids are involved in the reduction in firing rate of oxytocin neurones (Douglas et al., 1995). It is not known if the opioid mechanism involved in pregnancy-induced hypoalgesia is restricted to the spinal cord or is also active in the brain. If a brain opioid pathway was involved then this could provide a route for excessive noxious stimulation of the afferent nerves of the uterus and cervix to affect oxytocin release. For the progress of parturition, oxytocin must be released to allow expulsion of the foetuses, and is also involved in maternal behaviour (Fahrback et al., 1985), and therefore inhibition of oxytocin may compromise the welfare of both the sow and her piglets.

In conclusion, this study provides evidence that, in the sow, an endogenous analgesic mechanism(s) is activated during late pregnancy and parturition. This appears to be mediated, at least in part, via an opioid mechanism during parturition. The effect of the resulting opioid activity as a negative feedback to oxytocin release should be investigated.

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The effect of environment on behavioural activity, ACTH, β-endorphin and cortisol in pre-farrowing gilts

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Abstract

This study examined the temporal relationships between behavioural activity and hormones associated with stress in gilts farrowing in two environments. Thirty-one Large White × Landrace gilts with indwelling jugular catheters were blood sampled daily (08.00 and 16.00 h) from 10 days before their expected parturition date (EPD). Five days before EPD they were moved to either a farrowing crate (C) with no bedding, or a pen (P) (2·5 m × 3·0 m) with straw provided and were blood sampled daily at 08.00, 12.00 and 16.00 h. Around 12 h before the onset of farrowing an extension was fitted to the catheter and blood samples were taken remotely at 30-min intervals. The posture of the gilts was recorded using 5-min scan samples over the 24 h pre-farrowing. The proportion of scans standing (an index of activity) was strongly affected by time (P < 0·001) with peak levels at approximately 8 h pre-farrowing in both treatments, and by treatment (0·25 v. 0·33 (s.e.d. 0·03) for C and P gilts respectively; P < 0·05). Plasma cortisol concentrations also increased before farrowing (P < 0·001) reaching a peak at 12 to 6 h pre-farrowing. Crated gilts had higher cortisol concentrations than penned gilts (overall mean 41·5 v. 30·7 (s.e.d. 3·8) μg/l for C and P gilts respectively; P < 0·05) at 24 to 12 (P < 0·05), 12 to 6 (P < 0·01) and 6 to 2 (P < 0·05) h pre-farrowing. Plasma ACTH concentration showed a similar pattern to cortisol over the pre-parturient period, peaking at 12 h pre-farrowing in both treatments (time: P < 0·001); crated gilts had significantly higher concentrations of ACTH at 6 h pre-farrowing only (P < 0·05). Plasma β-endorphin concentrations also showed a gradual rise (P < 0·001) towards parturition; however no treatment differences were seen. These results suggest that the pituitary-adrenal (PA) axis is stimulated during pre-farrowing activity irrespective of farrowing environment. Crates, without bedding, further stimulate the PA axis over the pre-farrowing period perhaps by preventing nest-building. The rise in β-endorphin may be involved in an endogenous defence against parturition pain.

Keywords: *farrowing, gilts, nesting, pig housing, pituitary-adrenal axis.*

Introduction

When pre-parturient pigs are given the opportunity, they will isolate themselves from the herd and select a site in which to build a nest. Nest-building in free-ranging pigs consists of digging a hole and placing grass and other soft materials into it; a process which can take several hours (Jensen, 1986). In commercial production sows commonly farrow in a crate environment. Pigs in these conventional farrowing crates continue to show behaviours indicative of nest-building during the pre-parturient period; an increase in activity is seen (Meunier-Salaun *et al.*, 1991) and also in floor and fixture-directed behaviour (Lawrence *et al.*, 1994). Nest-building behaviours appear to be performed by pigs in a barren environment and by naïve gilts suggesting that there is an internal stimulus. This is further reinforced by the continuation of nest-building behaviour in sows when presented with a pre-formed nest (Arey *et al.*, 1991).

A study by Castren *et al.* (1993) suggested a possible hormonal control in that the initiation of nest-building occurred due to the pre-parturient rise seen in prolactin. However a study by Lawrence *et al.* (1994) did not find a convincing relationship between

prolactin and substrate directed behaviour, with some gilts performing nest-building behaviour and showing no rise in prolactin.

In addition to an internal stimulus for nest-building, there are also external factors which can modify the behaviours performed. The substrates available to the sow are of great importance as when offered a choice of farrowing sites sows will always choose a strawed environment as their nesting site (Arey *et al.*, 1989). Repeated provision of sawdust to young sows causes an increase in activity and behaviours characteristic of nest-building (Cronin *et al.*, 1993), suggesting that substrates in the environment modify nest-building. However within conventional farrowing systems substrates are often lacking. It has been suggested that pre-parturient sows are not highly motivated to obtain straw (Hutson, 1988), however the sows used in this particular study were multiparous and had no prior experience of straw-bedding thus rendering it a novel substrate.

A number of studies have previously reported that cortisol rises in sows around parturition. However these studies were all conducted using conventional farrowing crate systems (Killian *et al.*, 1973; Molokwu and Wagner, 1973). In a comparative study contrasting the response of gilts to crates and pens, gilts housed in crates with no bedding had higher concentrations of plasma cortisol relative to gilts housed in straw-bedded pens over the pre-parturient period (Lawrence *et al.*, 1994). Within this study the crated gilts were also found to spend less time standing and more time lying laterally and they performed also more fixture-directed behaviour than penned gilts, perhaps due to lack of straw. One interpretation of these results is that increased concentrations of maternal cortisol in the pre-farrowing period reflect interference of nest-building by the crate rather than the parturition process itself.

Plasma cortisol is commonly used as an indicator of physiological stress and the aim of this study was to determine the relationship between plasma cortisol and activity of pre-parturient gilts in two environments. In addition, fully to test the hypothesis that crates without bedding specifically stimulate the pituitary-adrenal (PA) axis, measurements were extended to include plasma ACTH and β-endorphin which are released concomitantly from the pituitary (Guillemin *et al.*, 1977).

Therefore the overall aim of this study was to examine the effects of farrowing environment on stimulation of the PA axis and to investigate any relationship with changes in pre-parturient activity.

Material and methods

Animals

The subjects of this study were 32 Large White × Landrace primiparous females (gilts; Cotswold Pig Development Co. Lincoln, UK). The gilts were purchased, at approximately 6 months of age, in eight consecutive groups (no. = 4) and each group was housed in a straw-bedded pen (2·6 m × 4·1 m). The gilts were offered 2·5 kg commercial diet providing 13 MJ digestible energy (DE) per kg daily and the pens were cleaned as required with straw being provided for bedding twice a week. After a 4-week period a boar was introduced to the pen daily and the gilts were served on 2 days consecutively. The expected parturition day (EPD) was calculated as 114 days after the first service date. Once pregnancy was confirmed at around 32 days after service the gilts were housed in groups in a strawed yard (9·6 m × 6·0 m) where they were given 2·5 kg/day on the floor of the same commercial diet. Similarly the pens were cleaned and straw for bedding provided twice weekly.

Catheterization

All gilts had a jugular catheter (silastic, Osteotec Ltd, Christchurch, Dorset, UK; i.d. 1·47 mm and o.d. 1·93 mm) implanted under general anaesthesia around 15 days before the EPD (15 (s.e. 0·66) days) (for full details of the procedure see Lawrence *et al.*, 1992). Briefly, the catheter was protected with an adhesive neck bandage and a connecting tap at the back of the neck was used for the removal of blood. The catheters were flushed daily with saline and primed with heparinized saline (150 i.u. per ml) until sampling began 10 days before EPD. After the operation, the gilts were housed individually in straw-bedded pens (2 m × 2 m). The same commercial food was offered in two meals at 08.00 and 16.00 h (2·5 kg/day). One gilt was removed from the study due to a blocked catheter.

Experimental housing

Five days before EPD the gilts were weighed and moved to either a conventional farrowing crate (treatment C; no. = 15) 2·25 m in length, 0·45 m in width and 1·05 m in height) or to a pen (treatment P; no. = 16) 2·5 m × 3·0 m). The crate consisted of a solid floor with slatted dunging area at the back and no straw was provided. The pen had a solid floor which was sloped to allow drainage and straw was provided. Both environments had a creep area outwith the specified dimensions. The temperature of the housing was controlled (mean minimum temperature 15·16 (s.e. 0·05)°C), mean maximum temperature 18·24 (s.e. 0·05)°C) and the lights were on between 08.00 and 16.00 h. Dim lights were used to allow observation during the night. The gilts were offered 3 kg/day of a food that provided 13·75 MJ

DE per kg and contained 180 g protein per kg in two meals at 08.00 and 16.00 h. After parturition the food level was gradually increased to appetite. The pens were cleaned and fresh straw provided after the morning meal and the slatted area in the crates was also cleaned at this time.

Blood sampling

The blood sampling procedure (Table 1) began 10 days before EPD (day –10) and continued until 5 min after the whole body of the first piglet (P1) had been expelled by the gilt. One sample was also taken 2 days after farrowing. Samples were collected directly from the tap at the neck until the onset of nest-building behaviours when a silastic extension tube was fitted. The catheter extension minimized the disturbance to the gilts by allowing samples to be taken from outside the crate or pen area. Saline was used to replace the volume of blood taken and the catheter and extension primed with heparinized saline (75 i.u. per ml). Heparinized monovette tubes (Sarstedt, Leicester, UK) were used to collect blood samples. The samples were kept at 4°C for 30 min before centrifuging. They were then spun at 3000 r.p.m. at 4°C for 20 min. Aliquots of plasma were pipetted and stored at –20°C for later assay.

Table 1 *Description of samples used in time periods for (a) cortisol and β-endorphin and specific time points for (b) ACTH. pr-m = pre-move: baseline samples taken before movement into the allocated farrowing environments, po-m = post-move: samples taken on the day of the move (day –5) to the allocated farrowing environment, m + 1 = samples taken during the day after the move to the allocated environment. P1 = birth of the first piglet. Numbers in time columns relate to hours pre-farrowing*

Time	Blood samples used in analysis
(a) Cortisol and β-endorphin	
pr-m	Day –10 16.00, Day –6 12.00, 16.00
po- m	Day –5 12.00, 16.00
m + 1	Day –4 08.00, 12.00, 16.00
–48/–24	4-h samples (08.00, 12.00, 16.00)
–24/–12	4-h samples (08.00, 12.00, 16.00)
–12/–6	Every 30 min
–6/–2	Every 30 min
–2/–1	Every 10 min
–1/0	Every 10 min
0	P1 and P1 + 5 min
(b) ACTH	
pr-m	Day –6 12.00
–48	–48 h
–24	–24 h
–12	–12 h
–10	–10 h
–8	–8 h
–6	–6 h
–4	–4 h
–2·5	–2·5 h
–1	–1 h
+2 days	12.00 on day +2

Hormonal analysis

Blood samples from different groups and treatments were balanced across assay runs. Plasma used for all assays had been thawed once only.

Cortisol. Cortisol was extracted using diethyl ether from 100 µl of plasma and concentrations were measured using radioimmunoassay of the extracted steroid (Duncan *et al.*, 1990). The intra- and inter-assay coefficients of variation were 0·1125 and 0·1496 respectively and the minimum detectable level of the assay was 0·23 µg/l. Single samples were extracted and then assayed in duplicate.

β-endorphin. Aliquots of plasma (200 µl) were assayed in duplicate using radioimmunoassay. Dextran-charcoal was used in the separation process and the supernatant counted using a multigamma counter (Ebling and Lincoln, 1987). Intra- and inter-assay coefficients of variation were 0·1504 and 0·1273 respectively and the minimum detectable level of the assay was 49 ng/l.

ACTH. ACTH concentrations were measured in single aliquots of 100 µl of plasma using an immunoradiometric assay kit (Euro Path Ltd, Bude, Cornwall, UK). Second antibody method was used, the samples spun and the pellet counted using a multigamma counter (Brooks, 1992). The minimum detectable level of the assay was 5·0 ng/l and the intra-assay coefficient of variation was 0·0832.

Behavioural observations

During the 48 h before the EPD the behaviour of individual gilts was recorded on 24-h time-lapse video to enable general activity to be recorded. The posture of the gilts was recorded at 5-min intervals during the 24 h pre-farrowing. Four categories of posture were recorded: stand, sit, lie ventrally and lie laterally.

Statistical analysis

Hormonal measures. The results of the assays were divided into time periods (Table 1 a and b). The data were normalized by log transformation and analysed using a repeated measures analysis of variance (ANOVA; Genstat version 5), with a blocked structure for pig and time period. Factors were treatment (two levels — crate and pen), group (eight levels) and time period (10 levels for cortisol and β-endorphin (Table 1a), and 11 levels for ACTH (Table 1b)). Post-hoc analysis was carried out where a significant time or treatment effect, or a time × treatment interaction was found. Comparison between treatments was

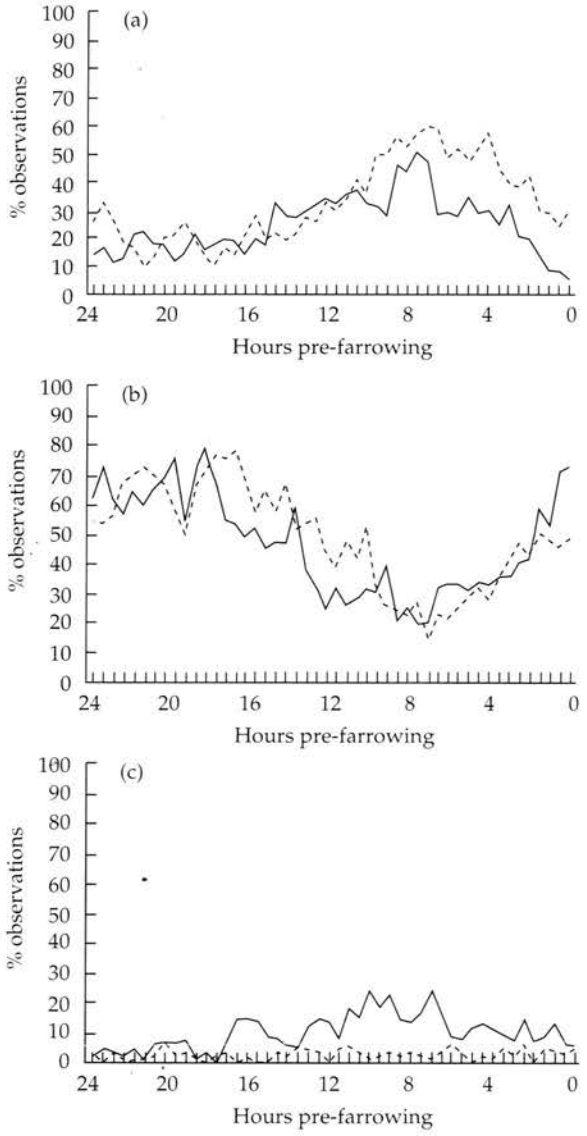

Figure 1 Changes in the percentage of observations spent (a) standing, (b) lying laterally and (c) sitting in crated gilts (——, no. = 15) and penned gilts (- - -, no. = 16) over the pre-farrowing period.

made by *t* tests (Minitab version 7.2) of mean values for each pig at individual time periods. When looking at time effects within treatments, paired *t* tests (Minitab version 7.2) were used to test whether the mean difference between each time period and the baseline (pre-move) was significantly different from zero.

Behavioural observations. Data obtained from the 5-min scan samples were pooled into 30-min time periods for analysis. The proportion of observations within each 30-min period was calculated for standing, sitting, lying ventrally and lying laterally. A repeated measures analysis of variance (ANOVA, Genstat 5) was used to analyse these data to determine treatment and time effects. Factors were treatment (two levels — crate and pen), group (eight levels) and time (48 levels).

Relationship between activity and hormonal levels. To determine whether there was any relationship between activity and hormonal levels a correlation of activity and cortisol and activity and β-endorphin was carried out for each individual gilt using data from all time periods. A *t* test (Minitab, version 7.2) was then used to determine whether the correlation coefficients for all the gilts were significantly different to zero and then a further *t* test was carried out to compare the correlation coefficients between the two environmental treatments.

Results

Gestation duration and weight of gilts

The average gestation length of the gilts was not affected by the farrowing environment (mean gestation length (days) 113 (s.e. 0·38) and 113·4 (s.e. 0·33) for crate and pen animals respectively). The average weight of the gilts on entrance to their allocated farrowing environment was 200·2 (s.e. 3·8) kg for crate animals and 200·6 (s.e. 3·0) kg for the animals in pens.

Behaviour

The posture of the gilts over the 24 h pre-farrowing showed changes as parturition approached. Standing was strongly affected by time ($F_{47,573}$ = 6·73, $P < 0·001$) and increased from about 16 h pre-farrowing in both treatments reaching a peak at 8 h pre-farrowing (Figure 1a). Crated gilts were observed standing for fewer of the observations (0·25 *v.* 0·33 (s.e.d. 0·03) for crate and pen gilts respectively) resulting in a treatment effect ($F_{1,15}$ = 5·52, $P < 0·05$). In contrast to standing, lying laterally decreased at around 16 h pre-farrowing and reached a low at about 8 h pre-farrowing (Figure 1b) (time effect: $F_{47,573}$ = 10·53, $P < 0·001$). Lying ventrally and sitting changed with time and sitting was also affected by treatment ($F_{1,15}$ = 52·56, $P < 0·001$) with crated animals sitting for a greater proportion of observations (0·10 *v.* 0·03 (s.e.d. 0·01) for crate and pen gilts respectively) (Figure 1c).

Cortisol

Before being moved to the farrowing environment circulating plasma cortisol concentrations did not differ between gilts allocated to the two environments but were elevated in both treatments

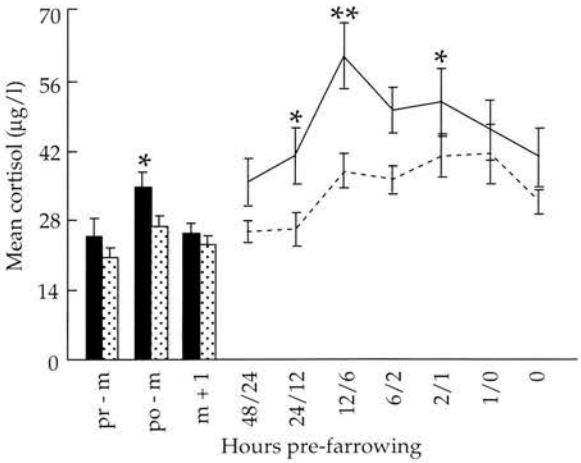

Figure 2 Mean plasma cortisol concentrations of crated (■, ——, no. = 15) and penned (⁘) - - -, no. = 16) gilts over the pre-farrowing period. pr-m = pre-move: baseline samples taken before movement into the allocated farrowing environments, po-m = post-move: samples taken at 12.00 and 16.00 h on the day of the move (day –5) to the allocated farrowing environment, m + 1 = samples taken during the day after the move to the allocated farrowing environment. Significance levels refer to differences between treatments at specific time periods.

following the move (see Figure 2), with the elevation of cortisol being greater in crated gilts (treatment: $t_{(25)} = 2{\cdot}09$, $P < 0{\cdot}05$). The increase seen in both crated and penned gilts was significant within treatment compared with pre-move values (see Table 2a). Cortisol concentrations fell in both treatments within 1 day of the move to concentrations which were not significantly different either between treatments or from pre-move levels.

A similar general trend in changes of plasma cortisol concentration was seen in both crated and penned gilts as parturition approached (time: $F_{9,119} = 18{\cdot}06$, $P < 0{\cdot}001$). However crated gilts showed higher levels of plasma cortisol than penned gilts during much of the pre-parturient period (see Figure 2); (treatment: $F_{1,15} = 7{\cdot}23$, $P < 0{\cdot}05$).

The crated gilts showed a non-significant rise in plasma cortisol concentrations at –24 to –12 h (Table 2) whilst levels in the penned animals remained steady, resulting in a significant difference between treatments at this time ($t_{(16)} = 2{\cdot}28$, $P < 0{\cdot}05$). Both treatments showed an increase in plasma cortisol concentration at –12 to –6 h (Table 2) but the rise was greater in crated gilts ($t_{(21)} = 3{\cdot}10$, $P < 0{\cdot}01$). Plasma cortisol concentrations in crated gilts started to decrease at –6 to –2 h but continued to remain

Table 2 *Changes in plasma cortisol, β-endorphin and ACTH concentration over the pre-farrowing period in relation to a baseline (**pre-move** = 100%) for the two environments. pr-m = pre-move: baseline samples taken before movement into the allocated farrowing environments, po-m = post-move: samples taken on the day of the move (day –5) to the allocated farrowing environment, m + 1 = samples taken during the day after the move to the allocated farrowing environment. Numbers in time columns relate to hours pre-farrowing*

	Cortisol (%)				β-endorphin (%)			
Time	Crate		Pen		Crate		Pen	
pr-m	100		100		100		100	
po-m	140	*	131	*	108		95	
m + 1	105		110		125		108	
–48/–24	135		124		87		121	
–24/–12	157		129		97		103	
–12/–6	247	***	188	***	147	*	127	
–6/–2	206	***	176	***	133	**	144	*
–2/–1	212	**	202	**	129	*	138	*
–1/0	189	**	204	**	136	*	140	*
0	164	*	155	**	165	*	177	**

	ACTH (%)			
Time	Crate		Pen	
pr-m	100		100	
–48	134	*	130	*
–24	169	*	163	
–12	366	***	256	**
–10	341	***	219	*
–8	321	***	238	**
–6	325	***	190	*
–4	310	***	197	*
–2·5	248	***	184	*
–1	268	**	208	**
+2 days	136	*	130	*

significantly higher in comparison with penned gilts ($t_{(22)} = 2{\cdot}61$, $P < 0{\cdot}05$).

Each treatment continued to have significantly higher than baseline concentrations of plasma cortisol until the birth of the first piglet (see Table 2) but there were no treatment differences over this time period.

ACTH

Plasma ACTH concentrations did not differ between treatments before the gilts were moved into their allocated farrowing environment (see Figure 3).

A similar general pattern of plasma ACTH can be seen in both treatments, with an increase from –48 h until reaching a peak at –12 h. From this peak at –12 h ACTH levels declined gradually until the onset of parturition (see Figure 3); (overall time effect $F_{10,213} = 20{\cdot}83$, $P < 0{\cdot}001$). Over the pre-farrowing period the

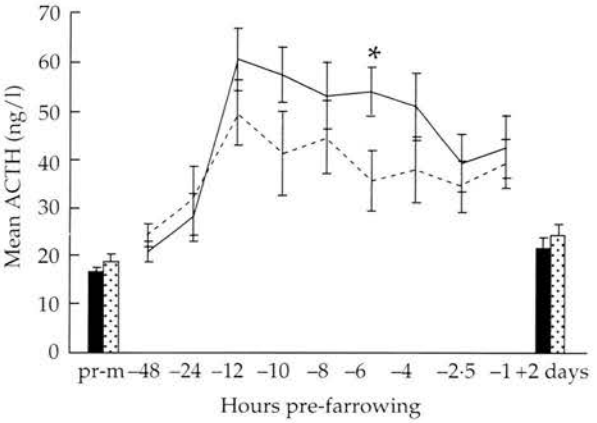

Figure 3 Mean plasma ACTH concentrations of crated (■, ——, no. = 15) and penned (⁘, - - -, no. = 16) gilts over the pre-farrowing period. pr-m = pre-move: baseline samples taken before movement into the allocated farrowing environments, +2 = a sample taken 2 days after farrowing. Significance levels refer to differences between treatments at specific times.

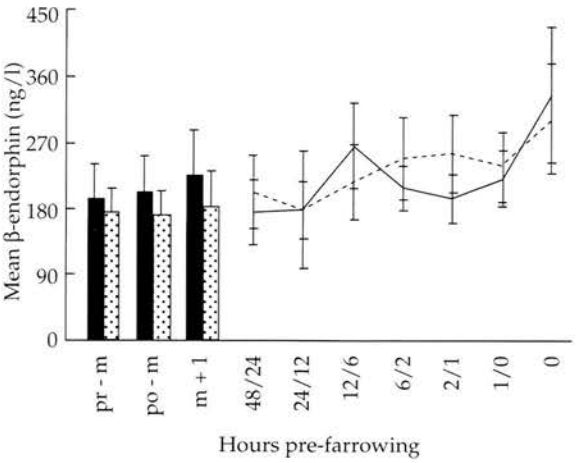

Figure 4 Mean plasma β-endorphin concentrations of crated (■, ——, no. = 15) and penned (⁘, - - -, no. = 16) gilts over the pre-farrowing period. pr-m = pre-move: baseline samples taken before movement into the allocated farrowing environments, po-m = post-move: samples taken at 12.00 and 16.00 h on the day of the move (day –5) to the allocated farrowing environment, m + 1 = samples taken during the day after the move to the allocated farrowing environment. Significance levels refer to differences between treatments at specific time periods.

crated gilts showed higher concentrations of plasma ACTH but this difference was not significant at the $P = 0{\cdot}05$ level. There was however a treatment effect on the pattern of plasma ACTH over time ($F_{10,213} = 1{\cdot}94$, $P < 0{\cdot}05$) with crated gilts having higher concentrations at –6 h ($t_{(22)} = 2{\cdot}23$, $P < 0{\cdot}05$).

A significant increase in plasma ACTH occurred by 48 h in both treatments compared with the baseline (pre-move). From –12 h until the birth of the first piglet, plasma ACTH concentrations were significantly higher than baseline concentrations within each environment but this was at a higher significance level in crated gilts (see Table 2).

A sample taken 2 days after parturition shows a fall of plasma ACTH concentration in both treatments to a level comparable with those seen 2 days pre-farrowing.

β-endorphin

Plasma β-endorphin concentrations gradually increased over the pre-parturient period in both treatments (time: $F_{9,95} = 12{\cdot}19$, $P < 0{\cdot}001$). A peak in plasma β-endorphin levels was seen in crated gilts at –12 to –6 h. However this was not significantly different from penned gilts at this time. A time × treatment interaction was found ($F_{9,95} = 2{\cdot}09$, $P < 0{\cdot}05$) but post-hoc analysis showed that plasma β-endorphin concentrations were not significantly different between treatments at any specific time period (see Figure 4). Both treatments showed a rise in β-endorphin levels at the birth of the first piglet.

By using pre-move levels as a baseline, significant increases were seen in crated gilts (Table 2), at –12 to –6 h which continued until the birth of the first piglet. The penned gilts did not show significant increases from the baseline levels until –6 to –2 h and this also continued until parturition (Table 2).

Relationship between activity and hormonal levels

Activity and β-endorphin. No significant relationship was found between β-endorphin and activity either overall (mean $r = 0{\cdot}109$, $P > 0{\cdot}05$ (no. = 31)), or when comparing treatments (mean $r = 0{\cdot}113$, $P > 0{\cdot}05$ and $0{\cdot}106$, $P > 0{\cdot}05$ for crate (no. = 15) and pen (no. = 16) gilts respectively).

Activity and cortisol. Changes in plasma cortisol and activity were positively correlated over the 24 h pre-farrowing (mean $r = 0{\cdot}23$, $P < 0{\cdot}01$ (no. = 31)), however when comparing treatments, only crated gilts showed a relationship between cortisol and activity (mean $r = 0{\cdot}28$, $P < 0{\cdot}05$ and $0{\cdot}18$, $P > 0{\cdot}05$ for crate (no. = 15) and pen (no. = 16) gilts respectively).

Discussion

As in other studies of pre-parturient behaviour in the pig (Meunier-Salaun *et al.*, 1991; Lawrence *et al.*, 1994), this study found that the time spent standing increased in all gilts irrespective of environment. The

proportion of time spent standing began to rise at around 16 h pre-parturition and reached a peak at 8 h pre-parturition, which was within the nest-building period defined by Castren *et al.* (1993). It has been suggested that nest-building behaviour is under internal control (Arey *et al.*, 1991; Castren *et al.*, 1993) as pre-parturient sows, when presented with a pre-formed nest, will continue to nest-build. Nest-building also appears to be modified by external stimuli as sows will choose a straw area, in preference to a hollow or a nest box, as their farrowing site (Arey *et al.*, 1989). This suggests that nesting material, as an external stimulus, is important to the pre-parturient sow and it has been shown that the absence of nesting material in crated sows increases the proportion of time spent sitting (Cronin *et al.*, 1993). Similarly, in the present study the proportion of time spent sitting was greater in the crated gilts, with no bedding, compared with those in a straw-penned environment. This posture may be an expression of the gilt's motivation, but inability, to perform nest-building, or alternatively, may be a posture that crated gilts adopt more frequently.

Crated gilts showed a greater response to introduction to the farrowing environments as they had significantly higher concentrations of plasma cortisol than penned gilts at this time. This may be due to the increased novelty of the crate, however this was short-lived as the elevated concentrations of cortisol had returned to a non-significant level within 24 h of the move. Over the 24 h pre-farrowing, which is the period of greatest activity, the crated gilts had elevated concentrations of plasma cortisol and ACTH. This physiological stress may have been due to the restrictive nature of the crate and the lack of substrate preventing the gilts from performing nest-building. The poor correlation between activity and plasma concentrations of cortisol observed in this study may have been due to the restriction of behaviour not resulting in immediate changes in hormonal levels. In the present study, both cortisol and ACTH increased irrespective of environment which, in terms of cortisol, is in contrast to a previous study by Lawrence *et al.* (1994). Two possible suggestions for this rise in cortisol and ACTH are that either the pre-farrowing period is stressful or that the straw-bedded pen used in this study was not an optimal environment for a pre-parturient gilt. Work by Jensen (1986) has shown that free-ranging pigs isolate themselves from the group in the 2 days before farrowing and normally select a covered area in which to build their nest. Therefore the elevated concentrations of plasma cortisol and ACTH seen in all gilts in this study may be partly accounted for by lack of isolation or cover.

As ACTH causes the release of cortisol from the adrenal cortex, it may be expected that the difference between the two environments would be similar in terms of plasma ACTH and cortisol. However, in this study there was much less of a difference between the plasma ACTH levels of the crated and penned gilts than that of plasma cortisol. Work in rats has shown that there is a change in the relationship between cortisol and ACTH during pregnancy with the amount of cortisol present, at a given concentration of ACTH, increasing towards parturition (Atkinson and Waddell, 1995). However this does not account for the treatment differences seen in the present study. A possible explanation may be that ACTH has been shown to have increased corticosteroidogenic activity resulting in elevated levels of cortisol, in rats in a stressful situation (Goverde *et al.*, 1993). Therefore in this study, plasma ACTH may have been rendered more biologically active by the stressful crate environment thus resulting in elevated plasma cortisol.

In this study plasma β-endorphin gradually increased in all gilts over the pre-farrowing period, reaching a peak at the birth of the first piglet. Plasma β-endorphin is known to increase in humans during labour (Facchinetti *et al.*, 1983; Fettes *et al.*, 1984; Hoffman *et al.*, 1984; Kofinas *et al.*, 1985; Fajardo *et al.*, 1994; McLean *et al.*, 1994) as does ACTH (Fettes *et al.*, 1984; Fajardo *et al.*, 1994). The rise in plasma β-endorphin may be involved in producing analgesia at peripheral sites in preparation for the onset of parturition. Peripheral sites for opioid analgesia have been demonstrated in the rat using paw inflammation (Joris *et al.*, 1987). Studies involving epidural anaesthesia (Raisanen *et al.*, 1984) and physical conditioning (Varrassi *et al.*, 1989) in pregnant women suggest that there is an inverse relationship between plasma β-endorphin and pain perception.

In conclusion, this study has demonstrated that the PA axis is stimulated during pre-farrowing activity irrespective of environment, which suggests that either the pre-farrowing period is stressful *per se* or that neither environment used in this study was optimal for a pre-parturient gilt. Further stimulation of the PA axis occurs in pre-parturient gilts housed in farrowing crates suggesting that, through the restrictive nature of the environment and the lack of substrate, reduced ability to perform nest-building behaviours causes physiological stress. The increased plasma opioid, β-endorphin, observed in gilts in both environments may be involved in the alleviation of pain during parturition by acting at peripheral sites.

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